Role of lipids in the diet of cultured and wild rock lobster larvae

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☼

Abstract

Food web interactions and appropriate forms of feed to promote growth and survivorship of phyllosoma larvae of rock lobsters in culture were examined. In particular, the role of key lipid nutrients has been investigated. These important steps are critical for closing the life cycle of rock lobster in aquaculture. Several protocols for enrichment of brine shrimp, *Artemia*, with essential polyunsaturated fatty acids (PUFA) were trialed. For the first time, *Artemia* were simultaneously enriched with essential PUFA [arachidonic (AA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids] in ratios specific to, and based on analyses of, wild phyllosomata. Newly-hatched phyllosomata of southern rock lobster, *Jasus edwardsii*, were fed enriched *Artemia* and on-grown to stage V in flow-through aquaria. Larvae decreased in total lipid from stages I–V. The major lipid class in all phyllosomata was polar lipid (PL), followed by sterol (ST), with no triacylglycerol (TAG) detected. Cultured phyllosomata had levels of AA and EPA similar to wild phyllosomata, although lower DHA.

To elucidate feeding capabilities, phyllosomata of packhorse lobster, *Jasus verreauxi*, were presented various food items and given chemical/tactile stimulation to induce feeding response. Phyllosomata are capable of processing hard prey items, and become entangled and fail to feed on soft tissue, such as jellyfish and mussel gonad. This represents the first documentation of the ability to process and ingest food by live phyllosomata.

Results from feeding trials, and analyses of potential prey items and pueruli, indicate that phyllosomata may require PUFA in PL form, which is largely unavailable via *Artemia*. Therefore, *J. edwardsii* phyllosomata were on-grown from newly-hatched to stage V, in static culture with antibiotics. Feeding larvae utilizing a PL-rich diet attached to mesh was compared to feeding with *Artemia*, enriched with a TAG-rich or an ethyl ester (EE)-rich nutrient source. For *Artemia*-fed phyllosomata, survival was high and total lipid remained generally constant to stage V. Both were notably higher than observed in previous feeding trials. The main fatty acids were oleic, linoleic, palmitic, EPA, stearic, *cis*-vaccenic, AA and DHA. Essential PUFA decreased from newly-hatched to stage V, although phyllosomata had absolute levels of essential PUFA greater than prior trials. The PL-rich diet displayed potential, as presence of faecal trails and molting confirmed that phyllosomata were consuming the diet.

This thesis has furthered understanding of phyllosomata physiology in challenging traditional ideology that larvae require PUFA in TAG form (i.e. *Artemia*). It demonstrated that lipids and PUFA are important nutritional components in rock lobster larvae. Feeding lipid-enriched *Artemia* was proven successful in early stages. The development of diets suitable for later stage larvae, with emphasis on enhancing PL, is a new and promising approach. Advancement of these concepts will facilitate successful culture of rock lobsters.

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Classification

Superkingdom Eukarya
Kingdom Animalia
Phylum Arthropoda
Subphylum Crustacea
Class Malacostraca
Subclass Eumalacostraca
Superorder Eucarida
Order Decapoda
Suborder Macrura Reptantia
Infraorder Astacidea
Superfamily Astacoidea
Family Astacidae
Genus *Astacus*, *Austropotamobius*, *Pacifastacus*
Family Cambaridae
Subfamily Cambarellinae
Genus *Cambarellus*
Subfamily Cambarinae
Genus *Barbicambarus*, *Bouchardina*, *Cambarus*, *Distocambarus*, *Fallicambarus*, *Faxonellav*, *Hobbseus*, *Orconectes*, *Procambarus*, *Troglocambarus*
Subfamily Cambaroidinae
Genus *Cambaroides*
Superfamily Nephropoidea
Family Nephropidae
Subfamily Neophoberinae
Genus *Acanthacaris*
Subfamily Nephropinae
Genus *Eunephrops*, *Homarus*, *Metanephrops Nephrops*, *Thymopides*
Subfamily Thymopinae
Genus *Nephropides*, *Nephropsis*, *Thymops*, *Thymopsis*
Family Thaumastocheles
Genus *Thaumastocheles*, *Thaumastochelopsis*
Superfamily Parastacoidea
Family Parastacidae

Genus *Astacoides*, *Astacopsis*, *Cherax*, *Engaeus*, *Engaewa*, *Euastacus*, *Geocharax*, *Gramastacus*, *Paranephrops*, *Parastacoides*, *Parastacus*, *Samastacus*, *Tenuibranchiurus*, *Virilastacus*

Infraorder Palinura

Superfamily Eryonoidea

Family Polychelidae

Superfamily Glypheoidea

Family Glypheidae

Genus *Neoglyphea*

Superfamily Palinuroidea

Family Palinuridae

Genus *Linuparus*, *Palinustus*, *Projasus*, *Puerulus*

Genus *Jasus*

e.g., *caveorum*, *edwardsii*, *frontalis*, *lalandii*, *paulensis*, *tristani*

Genus *Palinurus*

e.g., *delegoae*, *elephas*, *gilchristi*, *vulgaris*

Genus *Panulirus*

e.g., *argus*, *cygnus*, *interruptus*, *japonicus*, *longipes femoristriga*, *ornatus*, *penicillatus*, *polyphagus*

Genus *Sagmariasus*

i.e., *verreauxi* (Booth & Webber, 2001)

Family Scyllaridae

Genus *Palibythus*, *Palinurellus*

Family Synaxidae

Subfamily Arctidinae

Genus *Arctides*, *Scyllarides*

Subfamily Ibacinae

Genus *Evibacus*, *Ibacus*, *Parribacus*

Subfamily Scyllarinae

Genus *Scyllarus*

Subfamily Theninae

Genus *Thenus*

Infraorder Thallasinidea

Family Axianassidae, Callianassidae, Callianideidae, Laomediidae, Thalassinidae, Upogebiidae

Largely based on Holthuis (1991)

Authorities of Species

Artemia salina	Linnaeus, 1758
Brachionus plicatilis	Müller, 1786
Calycopsis borchgrevinki	Browne, 1910
Chaetoceros muelleri	Lemmermann, 1898
Chroomonas salina	(Wislouch) Butcher, 1967
Dicentrarchus labrax	Linnaeus, 1758
Euphausia superba	Dana, 1852
Homarus americanus	H. Milne Edwards, 1837
Isochrysis galbana (clone T. Iso)	Green
Jasus edwardsii	Hutton, 1875
Jasus verreauxi	H. Milne Edwards, 1851
Mytilus edulis	Linnaeus, 1758
Nototodarus sloanii	Gray, 1849
Panulirus cygnus	George, 1962
Panulirus interruptus	Randall, 1840
Panulirus japonicus	Vonsiebold, 1824
Panulirus ornatus	Fabricius, 1798
Paralichthys olivaceus	Temminck & Schlegel, 1846
Penaeus japonicus	Bate, 1888
Penaeus monodon	Fabricius, 1798
Penaeus semisulcatus	de Haan, 1844
Perna canaliculus	Gmelin, 1791
Sagmariasus verreauxi	proposed nomenclature change for *J. verreauxi* (Booth & Webber, 2001)
Salpa thompsoni	Foxton, 1961
Sparus aurata	Linnaeus, 1758
Stygiomedusa gigantea	Browne, 1910
Tetraselmis suecica	(Kylin) Butcher

Abbreviations

AA	arachidonic acid; 20:4(n-6)
BSTFA	N,O-bis-(trimethylsilyl)-trifluoroacetamide
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DAGE	diacylglyceryl ether
DG	diacylglycerol
DHA	docosahexaenoic acid; 22:6(n-3)
DM	dry mass
DPA	docosapentaenoic acid; 22:5(n-3)
EE	ethyl ester
EFA	essential fatty acid
EPA	eicosapentaenoic acid; 20:5(n-3)
FA	fatty acid
FAME	fatty acid methyl ester
FFA	free fatty acid
GC	gas chromatograph(y)
GE	1-*O*-alkyl glyceryl ether
HUFA	highly unsaturated fatty acid
LC	lipid class
LC-PUFA	long chain ($\geq C_{20}$) polyunsaturated fatty acid
LNA	linoleic acid; 18:2(n-6)
MDS	multidimensional scaling
MS	mass spectrometer(y)
MUFA	monounsaturated fatty acid
(n-x)	fatty acid with 1st double bond x carbon atoms from terminal methyl group. e.g., (n-3), (n-6)
NIWA	National Institute of Water and Atmospheric Research
OA	oleic acid; 18:1(n-9)c
OWL	oat bran–wheat germ–lecithin
PL	polar lipid
PUFA	polyunsaturated acid
RL	rock lobster
SD	standard deviation
SE	standard error
SFA	saturated fatty acid
ST	sterol
TAFI	Tasmanian Aquaculture and Fisheries Institute
TAG	triacylglycerol
TFA	total fatty acid
TLC–FID	thin-layer chromatography–flame-ionization detector
T_n	time/day *n*
TR	trace
TSE	total solvent extract
WE	wax ester
WM	wet mass

Preamble

Even without knowing a thing about marine ecology or catch fisheries, most people realize that lobsters have some value, even if that value is the massive dent in their wallet after enjoying "surf & turf". In fact, when you inform someone you are performing research with lobster, the first question they ask is, "Do you get to eat them?" Next, the main point of contention is which tastes best, whether it be clawed or rock/spiny lobster, warm or cold water species, claws, legs or tails, steamed or broiled, butter, sauce, red wine or white. Valid points. Those that are involved in some aspect of the industry, from fishers to wait staff, place a monetary value on lobster. These animals are a commodity, indirectly exchangeable for home mortgages and prams. Of course, there are those curious about the science of lobster; what makes them tick, what they get up to in their spare time. This thesis attempts to address all these points and dilute the skepticism of lobster aquaculture, even if more heavily weighted to one aspect than to another (good thing you read the abstract). So, "Yes, I do get to eat these decidedly delicious decapods" and enjoy southern rock lobster butterflied and grilled, with clarified butter, garlic and tarragon, and a chilled bottle of Tasmanian Pinot Noir. Any further questions?

☼

Chapter One

Introduction

Rock lobsters (RL) are found worldwide; their global distribution mirrored by growing international interest. *Jasus* species are only present in the Southern Hemisphere. Of 48 species of RL, the packhorse lobster, *Jasus verreauxi* H. Milne Edwards, 1851, is by far the most massive, with reports of animals reaching 20 kg and 70 cm total length. *J. verreauxi* is distributed along the southeast of Australia and New Zealand. The southern RL, *Jasus edwardsii* Hutton, 1875, has a range that extends to Western Australia, and can reach 8 kg and 60 cm (Booth & Webber, 2001).

To avoid predators such as octopuses (Berger & Butler, 2001), adult RL in the wild inhabit dens (Trendall & Bell, 1989; Lozano-Alvarez & Briones-Fourzan, 2001) and prey upon molluscs (Joll & Phillips, 1984; Juanes, 1992; van Zyl *et al.*, 1998), crustaceans and urchins (Goni *et al.*, 2001), sponges and algae (Joll & Phillips, 1984; Barkai *et al.*, 1996), and fish remains (Diaz-Arredondo & Guzmán del Proo, 1995). In culture they will also consume mussels (James & Tong, 1998), squid (Crear *et al.*, 2002), urchins and abalone (Mayfield *et al.*, 2001) and pellets (Crear *et al.*, 2002; Sheppard *et al.*, 2002), providing perspective into nutritional requirements (Bordner *et al.*, 1986; Smith, 1998) and feeding behavior (Zimmer-Faust & Case, 1982; Zoutendyk, 1988; Derby *et al.*, 2001; Sheppard *et al.*, 2002; Thomas *et al.*, 2002, 2003).

J. edwardsii females are sexually mature by 88 mm carapace length (MacDiarmid, 1989). After egg extrusion and a five month incubation, the eggs hatch during October and November at sunrise (MacDiarmid, 1985) as small, spider-like, transparent phyllosoma larvae. For phyllosomata, there is limited detail concerning natural prey (Shojima, 1963; Thomas, 1963; Nichols *et al.*, 2001) and behavior (Ritz, 1972a, b), and as reviewed by Cox & Johnston (2003c), regarding feeding capabilities (Batham, 1967; Kittaka, 1994a; MacMillan *et al.*, 1997). Fortunately, current research is endeavoring to explain phyllosoma feeding biology and function (Cox & Bruce, 2003; Cox & Johnston, 2003a, b). Unlike *Homarus* sp. (Harding *et al.*, 1991; Waddy & Aiken, 1995; Beal & Chapman, 2001) and *Thenus* sp. (Mikami

& Greenwood, 1997), the larval development in RL is quite long, as exemplified by *J. edwardsii* phyllosomata. These leaf-bodied larvae are planktonic and involve 11 distinct morphological stages and up to 17 molts, with an estimated maximum of two years before energy reserve-dependent metamorphosis to the benthic puerulus stage (Lesser, 1978; Phillips & Sastry, 1980; Booth, 1994; McWilliam & Phillips, 1997). Pelagic pueruli of *J. edwardsii* are non-feeding and swim significant distances from the open ocean prior to settlement in shallow coastal waters (Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a; Jeffs *et al.*, 2001b), settling down to a depth of 50 m (Booth *et al.*, 1991). Settlement cues are unclear, although the observed attraction of pueruli to a power station (Booth, 1989) may indicate sound. Also, pueruli are more likely to settle with conspecifics and refuge in conditioned, horizontal apertures (Booth, 2001).

Aquaculture

There is considerable interest worldwide in lobster aquaculture. In Australasia, RL has attracted interest as a potentially valuable aquaculture species. The fishery for *J. edwardsii*, boasts a value of over A$140 million in Australia (Punt & Kennedy, 1997; Hobday & Punt, 2001) and NZ$100 million in New Zealand (Breen & Kendrick, 1997). As catch fishery pressure escalates (Booth & Phillips, 1994), future exploitation of the RL marketplace will logically be realized through aquaculture (Phillips & Liddy, 2003).

In Tasmania (*J. edwardsii*), Western Australia (western RL, *Panulirus cygnus* George, 1962), Queensland (tropical RL, *P. ornatus* Fabricius, 1798) and New Zealand (*J. verreauxi* and *J. edwardsii*) there has been interest in the potential for the development of an aquaculture industry by initially capturing benthic pueruli or juveniles from the wild (Hayakawa *et al.*, 1990; Booth *et al.*, 1991; Jernakoff *et al.*, 1993; Guzmán del Proo *et al.*, 1996; Briones-Fourzan & McWilliam, 1997; Montgomery, 2000; Gardner *et al.*, 2001; Phillips *et al.*, 2001) and on-growing to market size. This form of aquaculture is sparking significant unrest within the catch fishery, especially concerning the methods of compensation for removal of recruitment stock. The concern is whether pueruli can be collected from the wild, while maintaining biological neutrality. Considering these factors, the long-term viability and sustainability of RL depends on closing the life cycle and developing an economically viable method of

raising lobsters from eggs through to market size. It is foreseen that aquaculture will need to continue with phyllosomata. After molt to pueruli, animals can be on-grown intensively (Matsuda *et al.*, 2001) or in sea-cages (Jeffs & James, 2001). In addition, the potential exists for aquaculture to provide stock for enhancement of wild fishery, as is the strategy with *Homarus* (Waddy & Aiken, 1995; Castro *et al.*, 2001; Jorstad *et al.*, 2001; Beal *et al.*, 2002). However, the complex life cycle of RL has been to date a major hindrance in aquaculture.

To meet the challenge, several aspects vital to aquaculture can be identified. As with all intensive aquaculture, these aspects include temperature (Matsuda & Yamakawa, 1997; Tong *et al.*, 2000a), light (Moss *et al.*, 1999; Chen *et al.*, 2001), salinity (Smith *et al.*, 2003b), stocking density, and egg development and timing of hatch, for increased availability of larvae (Tong *et al.*, 2000b; Matsuda *et al.*, 2002; Smith *et al.*, 2002b; Smith *et al.*, 2003a). For culture of RL phyllosomata, the primary aspect is the lack of information on diet and feeding behavior, which has inhibited determination of appropriate format of feed presentation. Coupled with suitable aquarium design (Greve, 1968; Illingworth *et al.*, 1997; Kittaka & Booth, 2000; Ritar, 2001) and feeding regimes (Liddy *et al.*, 2003), the format should optimize exposure of animals to food (Ritar *et al.*, 2003b), while minimizing microbial loading (Igarashi *et al.*, 1990; Diggles *et al.*, 2000; Handlinger *et al.*, 2000; Diggles, 2001). By default, lobster aquaculture has resorted to brine shrimp (*Artemia*), a vestige of finfish aquaculture. As summarized in recent reviews of RL aquaculture, culture of phyllosomata to pueruli has been successfully achieved in Japan and New Zealand in limited numbers (Kittaka & Booth, 2000; Phillips & Liddy, 2003). Raising phyllosomata fed on *Artemia* or mussel (*Mytilus edulis*) tissue has been moderately effective (Kittaka, 1988; Kittaka & Ikegami, 1988; Kittaka *et al.*, 1988; Yamakawa *et al.*, 1989; Kittaka, 1997a, b; Kittaka & Abrunhosa, 1997; Matsuda & Yamakawa, 1997; Tong *et al.*, 1997; Moss *et al.*, 1999), but nutrition still appears to be the key for further research and development.

Lipids

Although information regarding the roles of proteins, carbohydrates, vitamins and inorganic elements is necessary, much less is know about lipid nutrition (Sargent *et al.*, 2002). Knowledge of lipid composition of marine organisms provides a basis for

understanding energy storage, buoyancy strategies, trophodynamic interactions and therefore nutritional requirements. The most common lipids, and thus most often studied, are fatty acids (FA), sterols (ST), triacylglycerols (TAG), phospholipids and wax esters (WE) (Nevenzel, 1970; Lee *et al.*, 1972; Benson & Lee, 1975; Sargent *et al.*, 1976; Sargent, 1978; Lee & Patton., 1989). Over 120 ST have been isolated in marine invertebrates (Goad, 1978), although the major ST for most marine species is cholesterol (Tucker, 1989). Diacylglyceryl ether (DAGE) is generally a less common neutral lipid in marine organisms, and has been reported to occur in pteropod molluscs at up to 41% of total lipid (Phleger *et al.*, 1997; Kattner *et al.*, 1998). Lipids may be synthesized in part by marine animals, although some are derived only from diet and are considered essential, as is the case of specific polyunsaturated fatty acids (PUFA) in abalone (Uki & Watanabe, 1992; Nelson, 1999). Evidence implies a similar essentiality for long-chain PUFA in adult RL (Kanazawa & Koshio, 1994; Glencross *et al.*, 2001) and other crustaceans (Castell & Covey, 1976; Dall, 1981; Boghen & Castell, 1982; Kean *et al.*, 1985; Chandumpai *et al.*, 1991; Conklin *et al.*, 1991; Hopkins *et al.*, 1993; Saski *et al.*, 1996; Glencross *et al.*, 1998). This thesis examines the long-chain PUFA that are likely to be considered essential to RL phyllosomata: arachidonic acid [AA, 20:4(n-6)], eicosapentaenoic acid [EPA, 20:5(n-3)] and docosahexaenoic acid [DHA, 22:6(n-3)]. Clarification of lipid class and component FA requirements of phyllosomata will be a key to successful RL aquaculture.

Objective

Research described in this thesis attempts to identify key lipid nutrients vital to improving growth and survivorship of cultured Australian RL. This important step is essential for closing the life cycle of RL in aquaculture. The focus has been the examination of lipids in different stages of RL life cycle, including phyllosoma, puerulus and adult. Analyses of lipids included lipid extraction and quantification of lipid classes, FA, ST and 1-*O*-alkyl glycerols. The lipid classes examined were WE, DAGE, TAG, free fatty acids (FFA), ethyl esters, diacylglycerols, ST and polar lipids (PL). A central part was the examination of PUFA. The progression of this thesis stems from preliminary research on wild phyllosomata of *J. edwardsii* (Appendix 1), with the premise that lipid and FA profiles of wild animals should be the basis for formulating aquaculture diets. A number of differing protocols for enrichment of

Artemia with essential PUFA were trialed (Chapter 2, Appendix 3). In addition to results of wild phyllosomata, the analytical results of pueruli of *J. edwardsii* (Appendix 1) and *J. verreauxi* (Appendix 2) were the first hints that during the life cycle of rock lobster there may be dramatic shifts in lipid requirements. A trial was then conducted feeding TAG-enriched *Artemia* to *J. edwardsii* phyllosomata (Chapter 3). Results from the feeding trial and from analyses of potential prey items indicated that phyllosomata may require lipid in a PL form. Since *Artemia* convert their lipid enrichment to TAG and are notorious for retroconverting DHA to EPA (Barclay & Zeller, 1996; Navarro *et al.*, 1999), the next step was to examine the feeding capabilities of phyllosomata, which was done with *J. verreauxi* (Chapter 4). The observations of phyllosomata consuming static food items, with the need to supply phyllosomata with a PL-rich diet, prompted another feeding trial which presented phyllosomata a diet on a feed station (Chapter 5). In addition, a feeding trial using enriched *Artemia* were conducted with *P. cygnus* (Appendix 4), enabling interspecies comparison. Assessment of wild caught phyllosomata, potential wild prey items and cultured phyllosomata have elucidated differences in lipid content and composition and identified key FA molecules. The resultant information can be used in the future to include specific lipids into formulated diets for aquaculture of this highly valuable and intriguing marine species.

Chapter Two

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The Effect of Diet on the Biochemical Composition of Juvenile *Artemia*: Potential Formulations for Rock Lobster Aquaculture

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Abstract

The lipid class and fatty acid (FA) composition of juvenile *Artemia* fed continuously on four diets—the microalga *Tetraselmis suecica*, a mix of oat bran–wheat germ–lecithin (OWL), OWL–eicosapentaenoic acid (EPA) and OWL–EPA–arachidonic acid (AA)—were examined over a 9-d experiment in an attempt to approximate the FA profile of phyllosoma larvae of wild southern rock lobster, *Jasus edwardsii*. The main difference in lipid class composition of *Artemia* fed the four diets was the relative level of polar lipid (PL) and triacylglycerol (TAG). By day 9, the algal-fed *Artemia* were highest in PL (95% of total lipid) and lowest in TAG (2%), whereas the remaining diets resulted in *Artemia* with 16–30% PL and 41–82% TAG. After 2 d, the relative FA composition of all *Artemia* treatments closely reflected those of the diets, with no marked change after further feeding (to day 9). In terms of the content of essential polyunsaturated fatty acids (PUFA), by day 5 *Artemia* fed: 1) with the algal diet contained 7 mg/g FA dry mass (0.3% DHA, 6.3% EPA, 3.4% AA of total FA); 2) with the OWL diet contained 3 mg/g (0.3% DHA, 0.9% EPA, 0.7% AA); 3) with the OWL–EPA diet contained 55 mg/g (6.2% DHA, 11.6% EPA, 1.1% AA); and 4) with the OWL–EPA–AA contained 83 mg/g (3.8% DHA, 7.5% EPA, 17.4% AA). The PUFA profiles of *Artemia* using the OWL–oil diets were similar to wild rock lobster phyllosomata, although levels of docosahexaenoic acid (DHA) were lower (10% DHA) than in *J. edwardsii* larvae. On the basis of PUFA composition data alone, the results suggest the suitability of the OWL–oil

mixed diets for consideration for feeding to *Artemia* used in the culture of southern rock lobster larvae, particularly if the level of DHA can be further enhanced.

Artemia are important for feeding of larval marine animals in aquaculture. However, without an enrichment period prior to use, *Artemia* fail to provide a nutritionally balanced dietary source, especially for essential fatty acids (FA) which are necessary for survival and growth (McEvoy & Sargent, 1998; Narciso *et al.*, 1999). Methods and the effects of using various commercial enrichment media have been described (McEvoy & Sargent, 1998; Sorgeloos *et al.*, 1998), although most techniques and products are designed for newly hatched *Artemia*, with 24 hrs of enrichment, and are formulated to meet the requirements of larval fish and prawns (Rees *et al.*, 1994; McEvoy & Sargent, 1998).

The southern rock lobster, *Jasus edwardsii*, has recently been identified as a potential species for aquaculture in Australia. The fishery for *J. edwardsii* is the second most valuable fishery in Tasmania, with a value of approximately AUS$40M (Punt & Kennedy, 1997). A major hindrance in the aquaculture of *J. edwardsii* has been the rearing of larvae, for which lipid nutrition is fundamental (Phleger *et al.*, 2001). The process of metamorphosing through 11 phyllosoma stages and to puerulus stages (Booth, 1994) is energetically demanding. During the last phyllosoma stage, energy reserves are accumulated (Lemmens, 1994) and are utilized by the nektonic puerulus (Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a; Jeffs *et al.*, 2001b; Jeffs *et al.*, 2002), which are non-feeding (Kittaka, 1990). In order for the larval transformation to occur, broodstock should be given a high quality diet (Smith, 1999) and phyllosomata must be provided with adequate lipid. To this end, the diet must supply phyllosomata with energy rich lipid, including the polyunsaturated fatty acids (PUFA) considered essential to crustaceans (Kanazawa *et al.*, 1979).

Through examination of wild and cultured phyllosomata, a basis has been established to enrich the *Artemia* with the PUFA eicosapentaenoic acid [EPA, 20:5(n-3)], docosahexaenoic acid [DHA, 22:6(n-3)] and arachidonic acid [AA, 20:4(n-6)] (Phleger *et al.*, 2001). DHA and EPA have been the focus of enrichments for *Artemia* used for finfish larvae (Léger *et al.*, 1987a; Rees *et al.*, 1994; Rasowo *et al.*, 1995; Evjemo *et al.*, 1997; Narciso *et al.*, 1999), although AA has recently been highlighted as a key element for *J. edwardsii* (Smith *et al.*, 2002a). One of the most

successful feeds to date for early stage phyllosomata has been *Artemia*, especially when grown to a size of approximately 1.5 mm over 5–9 d (Tong *et al.*, 1997; Ritar, 2001). There is a paucity of information available on enrichments for juvenile *Artemia* and the composition of enrichment media specifically designed to suit the requirements of rock lobster larvae. This study describes an experiment performed to manipulate the biochemical composition of *Artemia* over a 9-d growout cycle with a target PUFA profile designed to suit the requirements of *J. edwardsii* phyllosomata.

Materials and Methods

Artemia Culture

Decapsulated *Artemia* cysts (INVE, Great Salt Lake Prime Gold) were hatched at 28 ± 1 C in 50-L white fiberglass cones in 0.2-μm filtered brackish water (27 ± 1‰), with vigorous aeration and a 150-W globe suspended 0.5 m above the water surface to supply light. At 24 h, triplicate batches of 80,000 newly-hatched *Artemia* were drained from the hatching containers, rinsed in freshwater for 2 min and transferred to white plastic buckets containing 20 L of filtered seawater (0.2-μm filtered, 34 ± 1‰, 27 ± 1 C) at a density of 4/mL. Animals were grown for 9 d with one of four diets: 1) the microalga *Tetraselmis suecica*; 2) oat bran–wheat germ–lecithin (OWL, 50:6:4); 3) OWL–EPA [40% maxEPA (containing 18% EPA, 12% DHA); Martek Biosciences, USA]; and 4) OWL–EPA–AA [40% maxEPA-ARASCO (containing 40% AA), 1:1; Martek Biosciences, USA]. The maxEPA and ARASCO products were triacylglycerol-containing oils. *T. suecica* was cultured and the cell density was measured daily as described in Wilkinson (2000). The oat bran-based diets were prepared daily by homogenizing ingredients suspended in 500 mL of seawater in a household blender (high speed 15 min, Sunbeam, Australia), with large oat bran particles removed by a 63-μm screen sieve. The daily ration fed to *Artemia* increased from day 0–8: *T. suecica* (1.2×10^5–2.3×10^5 cells/mL) and other diets (0.023–0.192 g/L). The performance of each diet was assessed in terms of survival, growth, lipid and FA profiles of *Artemia*.

Artemia in each bucket were rinsed, re-suspended in filtered seawater and re-fed daily. Samples containing approximately 20,000 *Artemia* in 5 L were removed for analysis on days 2, 5 and 9. Triplicate *Artemia* samples were taken initially (0 hr) from the hatching cone before distribution of *Artemia* to treatments. *Artemia* were

counted and measured daily in triplicate subsamples from each replicate. Temperature, pH, dissolved oxygen and salinity were monitored twice daily. The environmental parameters remained stable for the duration of the on-growing period; salinity (35.7‰ ± 0.2), pH (8.3 ± 0.0), dissolved oxygen (9 mg/L) and temperature (26.9 C ± 0.1).

Lipid Extraction

Artemia and feed samples were filtered through 4.7-cm Whatman glass filters (GF/F) and rinsed with 0.5-M ammonium formate. They were lyophilized overnight prior to analysis. Samples were 5.6–263 mg dry mass, containing 0.8–50 mg lipid. Samples were quantitatively extracted overnight using a modified Bligh and Dyer (1959) one-phase methanol–chloroform–water extraction (2:1:0.8 v/v/v). The phases were separated by the addition of chloroform–water (final solvent ratio, 1:1:0.9 v/v/v methanol–chloroform–water). The total solvent extract (TSE) was concentrated using rotary evaporation at 40 C.

Lipid Classes

An aliquot of the total solvent extract was analyzed using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to quantify individual lipid classes (Volkman & Nichols, 1991). Samples were applied in duplicate to silica gel SIII chromarods (5-μm particle size) using disposable 1-μL micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The primary solvent system used for the lipid separation was hexane–diethyl ether–acetic acid (60:17:0.1), a mobile phase resolving non-polar compounds such as wax ester (WE), triacylglycerol (TAG), free fatty acids (FFA) and sterols (ST). A second non-polar solvent system of hexane–diethyl ether (96:4) was also used to resolve hydrocarbons, WE, TAG and diacylglyceryl ether (DAGE). After development, the chromarods were oven dried and analyzed immediately to minimize absorption of atmospheric contaminants. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, TAG (derived from fish oil), WE (derived from orange roughy oil) and DAGE (derived from shark liver oil); 0.1–10 μg range]. Peaks were quantified on an IBM compatible computer using DAPA Scientific software (Kala-

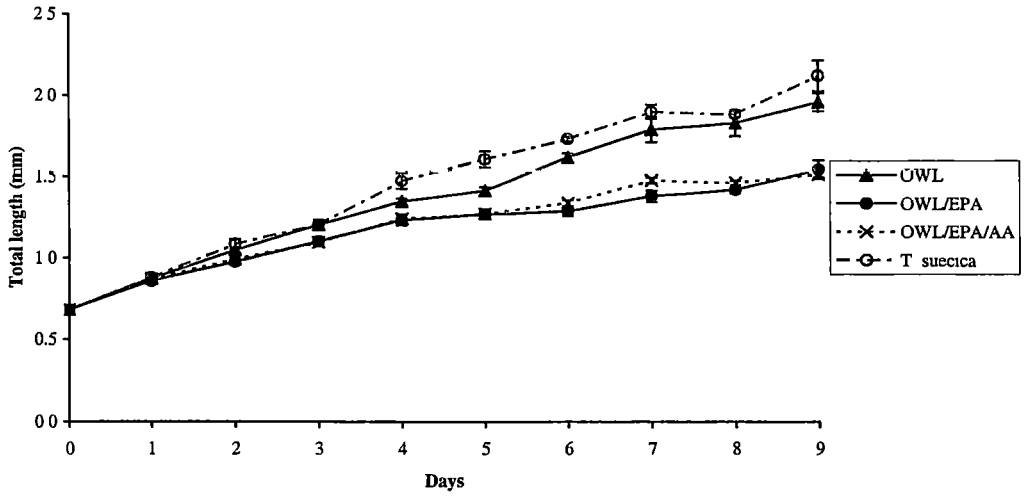

FIGURE 1. Artemia *size (mm total length) fed diets of* T. suecica, *oat bran–wheat germ–lecithin (OWL), OWL–eicosapentaenoic acid (EPA) and OWL–EPA–arachidonic acid (AA) for 9 d after hatch from cysts. Presented as mean ± SE.*

munda, Western Australia). TLC-FID results are generally reproducible to ±5–10% of individual class abundance (Volkman & Nichols, 1991).

Fatty Acids

An aliquot of the total lipid was *trans*-methylated to produce fatty acid methyl esters (FAME) using methanol–chloroform–conc. hydrochloric acid (10:1:1, 80 C, 2 hr). FAME were extracted into hexane–chloroform (4:1, 3 × 1.5 mL) and treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA 50 μL, 60 C, overnight) to convert ST and alcohols to their corresponding TMSi ethers.

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC (Avondale, PA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of methyl nonodecanoate and methyl tricosanoate internal standards, samples were injected in splitless mode at an oven temp of 50 C. After 1 min, the oven temperature was raised to 150 C at 30 C/min, then to 250 C at 2 C/min, and finally to 300 C at 5 C/min. Peaks were quantified with Waters Millennium software (Milford, MA, USA). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ

Table 1. Percentage lipid class composition and content of diets and on-grown *Artemia* fed different diets for 9 days.

	n	Wax esters	TAG	Free fatty acids	Sterols	Polar lipids	Lipid as mg/g dry mass
Diets							
Tetraselmis suecica	9	3.1 ± 1.9	2.3 ± 1.8	1.5 ± 1.8	1 7 ± 1.2	91.4 ± 4.4	109 ± 22
OWL	3	1.2 ± 0.8	30.5 ± 1.2	1.8 ± 0.6	0.7 ± 0.2	65.8 ± 1.3	288 ± 137
OWL–EPA	2	0.2 ± 0.0	76.5 ± 16.2	0.5 ± 0.2	1.3 ± 0.1	21.5 ± 16.1	404 ± 177
OWL–EPA–AA	2	0.6 ± 0.3	75.5 ± 12 7	0.7 ± 0.7	1.7 ± 0.0	21.6 ± 13.7	379 ± 260
Artemia							
Newly hatched	3	1.2 ± 0.3	53.5 ± 3.2	1.4 ± 0.4	1.7 ± 0.5	42.2 ± 2.3	230 ± 26
Tetraselmis suecica							
2 day	2	0.4 ± 0.1	2.5 ± 0.2	0.5 ± 0.1	3.8 ± 0.5	92.9 ± 0.4	197 ± 153
5 day	3	0.3 ± 0.2	4 3 ± 0.4	0.2 ± 0.1	4.6 ± 1.0	90.6 ± 0.8	98 ± 4
9 day	3	tr	0.7 ± 0.5	0.2 ± 0.1	4.1 ± 3.2	95.0 ± 3.7	94 ± 5
OWL							
2 day	3	0.7 ± 0.3	40.6 ± 17.6	0.9 ± 0.1	1.8 ± 0.5	56.0 ± 16.7	167 ± 43
5 day	3	0.1 ± 0.2	65.5 ± 5.6	0.8 ± 0.3	1.2 ± 0.3	32.4 ± 5.6	225 ± 36
9 day	3	tr	67.2 ± 6.0	1.4 ± 0.8	1.4 ± 0.6	30.0 ± 4.6	212 ± 10
OWL–EPA							
2 day	3	0.4 ± 0.1	61.5 ± 8.5	6.0 ± 1.8	1.1 ± 0.1	31.0 ± 8.5	291 ± 40
5 day	3	-	77.2 ± 5.3	2.7 ± 1.6	0.9 ± 0.5	19.2 ± 6.7	349 ± 7
9 day	3	0.1 ± 0.2	74.9 ± 10.1	1.3 ± 0.7	1.1 ± 0.4	22.5 ± 9.0	345 ± 17
OWL–EPA–AA							
2 day	3	0.6 ± 0.2	61.9 ± 1.2	6.8 ± 0.3	1.3 ± 0.1	29.4 ± 1.6	240 ± 7
5 day	3	0.9 ± 1.0	79.3 ± 3.3	0.9 ± 0.2	0.9 ± 0.5	17.9 ± 2.4	396 ± 29
9 day	3	0.1 ± 0.1	82 0 ± 6.9	0 9 ± 1.0	0.9 ± 0.4	16.1 ± 5.5	324 ± 17

Presented as mean ± sd; TAG, triacylglycerol; OWL, oat bran–wheat germ–lecithin, EPA, eicosapentaenoic acid; AA, arachidonic acid; (-), below detection; tr, trace.

GC-mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

Results

Artemia Growth

Survival of *Artemia* to day 5 remained high in all feeding treatments (≥95%). By day 9, survival of *Artemia* fed *T. suecica* or OWL remained high (95% and 85%, respectively), with survival for *Artemia* fed OWL–EPA or OWL–EPA–AA slightly lower (76% and 64%, respectively).

Artemia growth was affected by feeding treatment (Fig. 1). By day 2, *Artemia* fed *T. suecica* or OWL were longer (both 1.1 mm) than *Artemia* fed OWL–EPA or OWL–EPA–AA (both 1.0 mm) and this difference increased during the remainder of the *Artemia* feeding trial. By day 9, *Artemia* fed *T. suecica* or OWL were significantly longer (2.1 mm and 2.0 mm, respectively) than *Artemia* fed OWL–EPA (1.6 mm) or OWL–EPA–AA (1.5 mm).

Table 2. Percentage fatty acid composition and content of diets used for *Artemia* culture.

Fatty acid	*Tetraselmis suecica*[a]	OWL[b]	OWL–EPA[c]	OWL–EPA–AA[c]
14:0	0.5 ± 0.1	0.3 ± 0.0	5.2 ± 0.9	3.2 ± 0.2
C_{16} PUFA	3.0 ± 6.0	-	2.2 ± 0.5	1.6 ± 0.1
16:1(n-9)c	1.3 ± 0.3	-	0.1 ± 0.1	0.1 ± 0.0
16:1(n-7)c	2.0 ± 0.6	0.1 ± 0.0	6.1 ± 1.1	3.6 ± 0.3
16:1(n-7)t	tr	-	1.1 ± 0.1	0.7 ± 0.1
16:0	21.3 ± 4.1	19.4 ± 0.0	17.4 ± 0.0	15.0 ± 0.7
a17:0/17:1	0.3 ± 0.3	-	0.2 ± 0.0	0.1 ± 0.0
17:0	0.2 ± 0.3	-	0.4 ± 0.0	0.4 ± 0.0
18:3(n-6)	1.8 ± 0.5	-	0.1 ± 0.0	1.3 ± 0.1
18:4(n-3)	9.0 ± 1.9	-	2.1 ± 0.3	1.2 ± 0.0
18:2(n-6), LA	19.2 ± 5.2	42.1 ± 0.6	15.2 ± 7.7	14.5 ± 4.8
18:1(n-9)c/18:3(n-3)[d]	22.9 ± 4.5	30.5 ± 0.6	16.1 ± 0.1	14.8 ± 0.2
18:1(n-7)c	4.1 ± 2.2	1.2 ± 0.1	3.0 ± 0.3	2.0 ± 0.1
18:0	2.1 ± 2.4	2.9 ± 0.1	3.5 ± 0.1	5.5 ± 0.5
20:4(n-6), AA	1.5 ± 0.4	0.2 ± 0.0	0.8 ± 0.2	17.0 ± 2.3
20:5(n-3), EPA	5.1 ± 0.7	0.1 ± 0.0	12.6 ± 2.3	7.3 ± 0.6
20:3(n-6)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.1
20:1(n-11)c	1.3 ± 0.5	0.6 ± 0.0	1.0 ± 0.2	0.9 ± 0.1
22:6(n-3), DHA	0.2 ± 0.2	0.1 ± 0.1	7.8 ± 1.9	4.9 ± 0.6
22:5(n-3)	0.1 ± 0.2	-	1.6 ± 0.4	1.0 ± 0.1
22:1	1.8 ± 4.8	0.9 ± 0.2	0.6 ± 0.0	0.6 ± 0.0
Other	2.3	1.6	2.8	3.3
Sum SFA	25.6 ± 2.5	23.2 ± 0.2	27.8 ± 1.0	25.8 ± 0.3
Sum MUFA	33.6 ± 6.5	33.3 ± 0.8	28.5 ± 2.0	23.0 ± 0.5
Sum PUFA	40.7 ± 6.2	43.5 ± 0.9	43.6 ± 3.0	50.7 ± 0.9
Sum (n-3)	14.7 ± 2.0	0.6 ± 0.1	24.8 ± 5.0	14.9 ± 1.3
Sum (n-6)	23.0 ± 5.4	42.9 ± 0.8	16.6 ± 7.4	34.2 ± 2.3
Ratio (n-3)/(n-6)	0.64	0.01	1.49	0.44
Ratio EPA/AA	3.32	0.32	16.71	0.43
Ratio DHA/EPA	0.04	1.49	0.62	0.67
Total mg/g	78 ± 26	167 ± 47	312 ± 249	338 ± 237

Presented as mean ± sd; [a]$n = 9$; [b]$n = 3$; [c]$n = 2$; [d]18:1(n-9)c dominant; (-), below detection; tr, trace; OWL, oat bran–wheat germ–lecithin; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Other includes components present at <1%: i15:0, a15:0, 15:0, i17:0, 20:4(n-3), 20:2(n-6), 20:1(n-7)c, 20:0, 22:5(n-6), 22:4(n-6), 22:0, 24:1, 24:0.

Lipid Content and Composition

The OWL–EPA and OWL–EPA–AA diets contained more lipid (404 and 379 mg/g lipid dry mass, respectively; Table 1) than OWL (288 mg/g) or microalgae (*T. suecica*) (109 mg/g). The main difference between the feeds in terms of their lipid composition was the relative level of TAG and polar lipids (PL). The algal diet was highest in PL (91% of total lipid) and lowest in TAG (2%) and the OWL diet contained 66% PL and 31% TAG. In contrast, the OWL–oil diets contained lower PL

(22%) and high TAG (76%). All diets contained only low levels of FFA (0.5–1.8%). Also present as minor component in all diets were WE (0.2–3.1%) and ST (0.7–1.7%).

At hatch (T_0), *Artemia* had yolk sack present and contained 230 mg/g lipid. The lipid composition of these animals contained 54% TAG, 42% PL and <2% of each remaining lipid class (Table 1).

After 2 d of feeding (T_2), animals fed algae and OWL showed a decrease in lipid content (198 and 167 mg/g, respectively; Table 1). However, *Artemia* fed the OWL–oil diets increased in lipid content (OWL–EPA, 291 mg/g; OWL–EPA–AA, 240 mg/g). Relative to the T_0 *Artemia*, the T_2 *Artemia* fed algae or OWL contained lower TAG (2.5% and 41%, respectively) and higher PL (93% and 56%). The relative level of ST in the algae-fed *Artemia* was 3.8%, which was two to three fold greater than *Artemia* fed the other diets. On an absolute basis, however, the ST content was similar for *Artemia* fed the four diets (3–7 mg/g; Table 1). T_2 *Artemia* fed the OWL–oil mixtures increased in TAG (62%) and decreased in PL (30%). FFA levels of *Artemia* fed the OWL–oil mixtures increased to 6–7%, the highest of any *Artemia* samples during the experiment.

Many of the trends observed at T_2 continued to day 5 (T_5). Lipid content in algal-fed *Artemia* further decreased to 98 mg/g, and in OWL-fed *Artemia* increased to 225 mg/g and those fed the OWL–oil diets increased again to 349–396 mg/g (Table 1). *Artemia* fed the OWL and OWL–oil mixtures showed an increase in TAG (66–79% of total lipid) and decrease in PL (18–32%). The lipid class profile of the algal-fed *Artemia* was unchanged and remained dominated by PL.

At day 9 (T_9), lipid content of the *Artemia* was similar to results at T_5; no further increase occurred relative to T_5 (Table 1). *Artemia* fed the OWL–oil diets contained 324–345 mg/g lipid, with OWL-fed animals containing 212 mg/g and the algal-fed *Artemia* 94 mg/g. The relative levels of each lipid class for *Artemia* fed each diet were also similar to the T_5 *Artemia*.

Fatty Acid Composition

The principal FA components in *T. suecica* in order of decreasing abundance were: 18:1(n-9)c, 16:0, 18:2(n-6), 18:4(n-3), EPA (5.1%) and 18:1(n-7)c; these FA accounted for 82% of the total FA (Table 2). Of the essential long chain ($\geq C_{20}$)

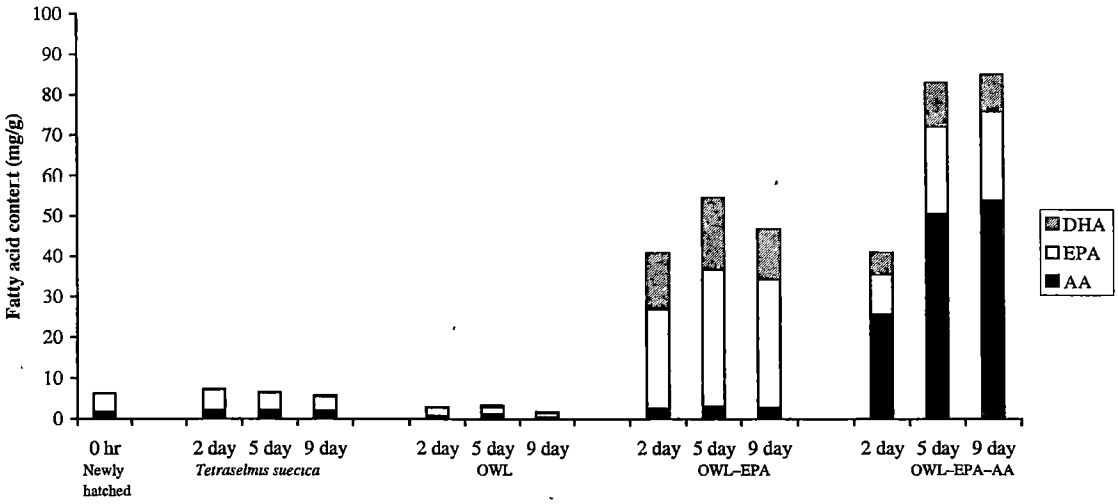

FIGURE 2. *Fatty acid content (mg/g) of the essential long chain-polyunsaturated fatty acids docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and arachidonic acid (AA) in newly-hatched* Artemia *and those fed diets of oat bran–wheat germ–lecithin (OWL), OWL–eicosapentaenoic acid (EPA), OWL–EPA–arachidonic acid (AA) or* T. suecica *for 9 d after hatch from cysts.*

(LC)-PUFA, DHA was absent and AA was only a minor component (1.5%). In the OWL diet, the major FA were 18:2(n-6), 18:1(n-9)c and 16:0; these three components accounted for 92% of the total FA. The level of PUFA was similar in the algal and OWL diets (40% and 44%). The essential LC-PUFA (AA, EPA and DHA) were only minor components in the OWL diet and were present at a combined level of only 0.4%. The OWL–oil diets contained similar levels of 16:0, 18:2(n-6), 18:1(n-9)c and other minor components such as 14:0, 16:1(n-7)c, 18:0 and 18:1(n-7)c (Table 2). The LC-PUFA profiles of the OWL–oil diets were similar to the source oils used; the OWL–EPA diet contained 12.6% EPA, 7.8% DHA and 0.8% AA, and the OWL–EPA–AA diet contained 7.3% EPA, 4.9% DHA and17.0% AA.

The major FA in T_0 *Artemia*, in decreasing order of abundance, were: 18:1(n-9)c, 16:0, 18:1(n-7)c, 18:4(n-3), 18:0, 18:2(n-6) and 16:1(n-7)c (84% of total FA; Table 3). T_0 *Artemia* contained only low levels of the essential PUFA; 2.4% EPA and 1.0% AA, with DHA absent. At T_2, the FA composition of all *Artemia* treatments closely reflected those of the diets used (Tables 2, 3). In terms of the essential LC-PUFA, algal-fed *Artemia* contained 6% EPA, 2.5% AA and 0.2% DHA. Animals fed OWL contained low levels of LC-PUFA; 1.4% EPA and 0.1% DHA, with AA present at 0.7%. T_2 *Artemia* fed the OWL–EPA diet contained higher EPA (10.4%), with 5.9% DHA and 1.1% AA. *Artemia* fed OWL–EPA–AA contained higher AA (16.9%), with 6.5% EPA and 3.6% DHA.

At both T_5 and T_9, the FA compositions of *Artemia* from all treatments were generally similar to the T_2 *Artemia* (Table 3, Fig. 2). Small increases were observed in the level of EPA in the OWL–EPA fed *Artemia* (T_2, 10.4%; T_5, 11.6%; T_9, 12.9%) and AA in the OWL–EPA–AA fed *Artemia* (T_2, 16.9%; T_5, 17.4%; T_9, 18.5%). DHA levels in the OWL–oil treatments increased slightly between T_2 and T_5, and then fell slightly at T_9. The enrichment of LC-PUFA was significantly greater using OWL–oil diets than with either the microalga *T. suecica* or OWL (Table 3). In fact, the mass of essential PUFA was 6–47 times greater in *Artemia* fed OWL–EPA (51–55 mg/g FA dry mass) and OWL–EPA–AA (41–85 mg/g) diets, when compared to the *T. suecica* (6–7 mg/g) and OWL (2–3 mg/g) diets (Fig. 2). There was no further enrichment of essential PUFA composition between days 5 and 9 (Fig. 2).

Discussion

We have demonstrated that, through the addition of LC-PUFA rich oils to the diet of *Artemia*, the LC-PUFA profile can be manipulated in a 2-d period, and that further on-growing did not change the profiles (on a percentage basis). The lipid and FA data presented here confirm the ability to design relatively simple and low cost *Artemia* diets with LC-PUFA profiles that are similar to those of wild-caught *J. edwardsii* phyllosomata (Smith, 1999; Phleger *et al.*, 2001), and may offer potential for use with the aquaculture of rock lobster larvae. The rock lobster requirements differ to those of larval finfish and prawns, as the larval stages contain high levels of PL and low TAG, are high in AA in early phyllosoma stages and exhibit a high DHA/EPA ratio in later phyllosoma stages (Smith, 1999; Phleger *et al.*, 2001). In addition, rock lobster phyllosomata enhanced growth with large (1.5 mm, 5–9 d) *Artemia* for optimal growth in culture (Tong *et al.*, 1997; Ritar, 2001).

The low levels of FFA in all diets (0.5–2%) indicated that they were stable under the time frame and conditions of the experiment. With reference to simulating phyllosomata lipid class profiles, we have demonstrated the ability to influence lipid class composition by increasing PL in *Artemia* fed algae and increasing TAG in those fed other diets. PL was also increased in *Artemia* fed microalgae in a study by Fan *et al.* (1998). The PL is similarly high in *J. edwardsii* phyllosomata (83–96%) (Phleger *et al.*, 2001).

High levels of the essential LC-PUFA were obtained with *Artemia* using simple diet mixes based on readily available commercial products. For the first time, juvenile *Artemia* were simultaneously enriched with all three essential PUFA, EPA, DHA and in particular AA. To put the quantitative results in a broader context, we compared the EPA/DHA/AA values to results obtained for other studies, the majority of which were a standard 24 hr enrichment of nauplii. For studies that examined the lipid composition of *Artemia* enriched for more than 48 hrs (Wache & Laufer, 1998; Zhukova *et al.*, 1998; Naegel, 1999; Thinh *et al.*, 1999; Olsen *et al.*, 2000), only three examined LC-PUFA enrichment (Wache & Laufer, 1998; Zhukova *et al.*, 1998; Olsen *et al.*, 2000). Of these studies, only Olsen *et al.* (2000) present LC-PUFA on an absolute basis. In that study, after 72 hrs of enrichment, the contents of EPA and DHA in *Artemia* were 14 mg/g and 4 mg/g, respectively (Olsen *et al.*, 2000). In our study, on-growing *Artemia* to 5 d on the OWL–EPA–AA diet proved more successful in boosting LC-PUFA content (83 mg/g; Table 3; Fig. 2), which, in comparison, is around four fold greater. Therefore, the feeding method described here may provide an alternative approach in comparison to conventional feeding protocols for larvae requiring 5-d *Artemia*, as is the case of rock lobster phyllosomata (Tong *et al.*, 1997; Ritar, 2001). Given the markedly larger size of the *Artemia* grown for 5 d (Fig. 1), the increase in LC-PUFA would be even greater when expressed as a per animal basis.

The relative level of linoleic acid [LA, 18:2(n-6)] present in the diets (Table 2) is reflected in the *Artemia* (Table 3). The level of LA increases from day 2 to day 9 with all diets, including the alga, and is greatest in *Artemia* fed the OWL diet (24–38% LA). Since LA is present at significant portions, it markedly changes the (n-3)/(n-6) ratio, which, for example, is low in the OWL-fed *Artemia* (0.02–0.14). The (n-3)/(n-6) ratio may be of physiological importance to larval health (Sargent, 1995; Smith *et al.*, 2002a), and with reference to rock lobster phyllosomata, this study demonstrates that it is easily possible to change the (n-3)/(n-6) ratio. This is very pertinent to *J. edwardsii* phyllosomata, where the (n-3)/(n-6) ratio changes through the stages in the wild (stage IV, ratio: 3.5; stage XI, ratio 5.8; (Phleger *et al.*, 2001). Therefore it will be possible to readily change the (n-3)/(n-6) ratio to suit the phyllosoma stage of rock lobster.

Table 3. Percentage fatty acid composition and content of *Jasus edwardsii* phyllosomata and newly-hatched *Artemia* and those on-grown for 9 days with different diets.

		Newly	*Tetraselmis suecica*			OWL			OWL–EPA			OWL–EPA–AA		
Fatty acid	Phyllosomata[a]	hatched	*2 day*[b]	*5 day*	*9 day*	*2 day*	*5 day*	*9 day*	*2 day*	*5 day*	*9 day*	*2 day*	*5 day*	*9 day*
14:0	3.2 ± 1.1	0.9 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	1.0 ± 0.0	1.2 ± 0.1	0.9 ± 0.0	2.6 ± 0.2	3.5 ± 0.5	2.6 ± 0.3	1.5 ± 0 1	1.9 ± 0.1	1.6 ± 0.3
C_{16} PUFA	0.1 ± 0.1	0.7 ± 0.1	1.1 ± 0.3	1.3 ± 0.9	0.3 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.6	0.3 ± 0.0	0.5 ± 0.0	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0 2 ± 0.0
16:1(n-9)c	1.3 ± 1.4	1.7 ± 0.0	0 8 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	1 1 ± 0.1	1.0 ± 0 0	1.5 ± 0.5	0.8 ± 0.0	0.7 ± 0.0	0.8 ± 0.1	0.6 ± 0.0	0.7 ± 0.2	0.6 ± 0.0
16:1(n-7)c	4.6 ± 1 6	3.5 ± 0.1	1.8 ± 0.1	1.5 ± 0.1	1.3 ± 0.0	1.9 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	4.9 ± 0.1	5.7 ± 0.5	5.5 ± 0.0	3.1 ± 0.1	3.7 ± 0.1	3.5 ± 0.1
16:0	16.2 ± 3.3	12.9 ± 0.1	12.1 ± 0.5	13.9 ± 0.3	11.3 ± 0.5	13.4 ± 0.1	13.9 ± 0.5	12.7 ± 0.1	14.1 ± 0.8	14.1 ± 1.0	12.6 ± 1.4	11.2 ± 0.5	10.5 ± 0.4	9.8 ± 1.5
a17.0/17:1	1.4 ± 0.4	1.7 ± 0.0	1.0 ± 0.0	0.3 ± 0 0	0.3 ± 0.0	0.8 ± 0.0	0.3 ± 0 0	0.2 ± 0.0	0.6 ± 0.1	0.4 ± 0 0	0.4 ± 0.0	0.5 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
17:0	0.8 ± 0.1	1.0 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0 5 ± 0.1	0.6 ± 0.1	0 5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.5 ± 0.1
18:3(n-6)	tr	1.7 ± 0.0	3.3 ± 2.8	1.8 ± 0.1	1.2 ± 0.0	0.6 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.4 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.0
18:4(n-3)	0.7 ± 0.2	5.2 ± 0.0	2.3 ± 3.3	4.5 ± 0.3	3.3 ± 0.1	1.8 ± 0.1	0.1 ± 0.2	0.1 ± 0.1	1.8 ± 0.1	1.4 ± 0.1	1.3 ± 0.0	1.4 ± 0.1	0.9 ± 0.1	0.8 ± 0.0
18:2(n-6), LA	4.0 ± 2.3	4.1 ± 0.8	10.0 ± 0.6	17.1 ± 0.5	16.6 ± 0.5	23.8 ± 2.0	33.8 ± 0.6	37.5 ± 2.9	10.7 ± 0.3	13.6 ± 0.2	16.2 ± 0.1	11.2 ± 0.1	14.8 ± 0.1	17.3 ± 0.3
18:1(n-9)c/18:3(n-3)[c]	27.3 ± 6.1	47.8 ± 0.8	33.2 ± 0.2	23.7 ± 0.1	23.1 ± 0.2	35.9 ± 1.7	31.4 ± 0.2	30.0 ± 1.7	27.6 ± 0.4	23.2 ± 0.3	22.9 ± 0.3	24.6 ± 0.5	20 8 ± 0 4	20.2 ± 0.6
18:1(n-7)c	4.8 ± 1.9	6.4 ± 0.1	10.1 ± 0.5	10.3 ± 0 5	12.0 ± 0.8	5.2 ± 0.3	3.5 ± 0.3	2.7 ± 0.3	5.7 ± 0.1	4.9 ± 0.1	5.4 ± 0.2	4.4 ± 0.2	3.7 ± 0.1	3.8 ± 0.1
18:0	7.7 ± 1.1	4.4 ± 0.0	8.7 ± 0.2	9.4 ± 0.3	11.8 ± 0.8	6.5 ± 0.2	6 1 ± 0.1	6 6 ± 0.4	5.1 ± 0.2	5.1 ± 0.5	5.6 ± 0.5	5.6 ± 0.1	5.5 ± 0.2	5 5 ± 0.9
20:4(n-6), AA	1.4 ± 0.5	1.0 ± 0.0	2.5 ± 0.1	3.4 ± 0.8	3.8 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	0.4 ± 0.2	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	16.9 ± 0.3	17.4 ± 0.6	18.5 ± 1.5
20:5(n-3), EPA	6 0 ± 2.3	2.4 ± 0.0	6.0 ± 0.1	6.3 ± 0.2	6.5 ± 0.7	1.4 ± 0.1	0.9 ± 0.1	0.6 ± 0.0	10.4 ± 0.2	11.6 ± 0 8	12.9 ± 1.5	6.5 ± 0.3	7.5 ± 0.2	7.5 ± 0.4
20:3(n-6)	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0 2 ± 0.0	0.1 ± 0.0	0 1 ± 0.0	tr	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	1.0 ± 0.1	1.0 ± 0 1	1 0 ± 0.1
20:1(n-11)c	1.5 ± 0.6	0.9 ± 0.0	1.2 ± 0.0	0.9 ± 0.0	0.7 ± 0.0	0.9 ± 0 0	0.8 ± 0.0	0.7 ± 0.0	1.4 ± 0.0	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	1 0 ± 0.1	0.8 ± 0.0
22:6(n-3), DHA	10 4 ± 4 4	tr	0.2 ± 0.0	0.3 ± 0.1	0.5 ± 0.2	0.1 ± 0 0	0 3 ± 0.0	0.2 ± 0.0	5.9 ± 0.2	6.2 ± 1.0	5.1 ± 0.7	3.6 ± 0.2	3.8 ± 0.1	3.1 ± 0.2
22:5(n-3)	0.3 ± 0 2	-	-	-	-	tr	-	-	1.5 ± 0.1	1.6 ± 0.3	1.3 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
Other	8.3	3.4	4.2	3.5	5.3	3.5	3.3	3.3	4.4	4.5	4.1	4.1	3.9	3.2
Sum SFA	32.1 ± 3.7	23.3 ± 0.2	25.1 ± 0.5	26.2 ± 0.6	26.9 ± 0.3	24.2 ± 0.4	23 5 ± 0.8	22.5 ± 0.7	24.5 ± 1.1	24.9 ± 2.3	23.1 ± 2.2	20.9 ± 0.6	20.0 ± 0.7	18.7 ± 3.0
Sum MUFA	45.4 ± 9.7	60.6 ± 0.8	47.8 ± 0.1	37.5 ± 0.3	38.8 ± 0.5	45.8 ± 1.8	39 0 ± 0.1	37.0 ± 2.4	41.9 ± 0.6	37.6 ± 0.4	37.1 ± 0.4	35.1 ± 0.7	31.1 ± 0.1	29.9 ± 0.4
Sum PUFA	23.8 ± 6.4	16.1 ± 0.7	26.7 ± 0.2	36.1 ± 0.6	33.5 ± 0.7	29.8 ± 2.0	37.6 ± 0 8	40.2 ± 3.0	33.5 ± 0.5	37.5 ± 2.5	39.7 ± 2.5	43.9 ± 1 2	48.9 ± 0.8	51.4 ± 2.7
Sum (n-3)	17.7 ± 6.9	8.2 ± 0.0	8.8 ± 3.2	11.3 ± 0 5	10.5 ± 0.5	3 5 ± 0.3	1.4 ± 0.3	0.9 ± 0.2	20.3 ± 0.3	21.4 ± 2.1	21.2 ± 2.3	12.9 ± 0.7	13.5 ± 0 4	12.7 ± 0.7
Sum (n-6)	6.1 ± 1.9	7.2 ± 0.8	16.7 ± 3.7	23.5 ± 0.3	22.7 ± 1.0	25.6 ± 2.1	35.5 ± 0.5	38 6 ± 2.7	12.8 ± 0.2	15.6 ± 0.4	18.1 ± 0.2	30.8 ± 0.4	35.0 ± 0.5	38.5 ± 1.9
Ratio (n-3)/(n-6)	2.90	1.15	0.53	0.48	0.46	0.14	0.04	0 02	1.58	1.37	1.17	0.42	0 39	0.33
Ratio EPA/AA	4.26	2.40	2.40	1.85	1.69	2.14	1.31	1.34	9.36	10.33	11.32	0.38	0.43	0.41
Ratio DHA/EPA	1.74	0.01	0.03	0.04	0 08	0.04	0.31	0.30	0.57	0.53	0.39	0.56	0.51	0.42
Total mg/g	nd	184 ± 3	85 ± 57	66 ± 5	53 ± 5	138 ± 9	189 ± 24	151 ± 57	235 ± 8	289 ± 32	245 ± 48	152 ± 12	291 ± 95	293 ± 18

Presented as mean ± sd; n = 3; [a]mean of stages III–V, n = 15, data from Phleger et al. (2001); [b]n = 2, [c]18:1(n-9)c dominant; (-), below detection; tr, trace; nd, not determined; OWL, oat bran–wheat germ–lecithin; LA, linoleic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Other includes components present at <1%· i15:0, a15:0, 15:0, 16:1(n-7)t, i17:0, 20·4(n-3), 20:2(n-6), 20:1(n-7)c, 20·0, 22:5(n-6), 22:4(n-6), 22:1, 22:0, 24:1, 24:0.

Juvenile *Artemia* fed the OWL–EPA diet have a similar LC-PUFA profile compared to wild early stage (I–V) phyllosomata of *J. edwardsii* (Table 3). This enrichment is also superior compared to other diets in terms of DHA incorporation (6 %; 18 mg/g). However, further DHA enrichment would be useful, as levels in wild phyllosomata reach 17% (Phleger *et al.*, 2001) and are even higher in potential prey items (up to 40%; unpublished data). The incorporation of EPA was successful in both OWL–oil diets, and for 5-d fed *Artemia*, was much higher in those fed OWL–EPA–AA (51 mg/g) and OWL–EPA (34 mg/g) than in those fed OWL (1.7 mg/g) or *T. suecica* (4 mg/g) (Table 3; Fig. 2). In *Artemia* fed the OWL–EPA–AA diet, we were also able to significantly boost the AA incorporation (Table 3; Fig. 2), which is particularly useful as AA has been identified as vital to nutrition in larval crustaceans (D'Souza & Loneragan, 1999; Smith *et al.*, 2002a). As yet the appropriate amounts of AA for inclusion in the diet have not been resolved. The juvenile *Artemia* fed OWL–EPA–AA demonstrate the ability to easily alter the percentage of AA. The capability to readily enhance AA levels in juvenile *Artemia* may prove important in aquaculture feed formulation for rock lobster larvae and other animals with similar requirements.

Feeding trials of phyllosomata using *Artemia* fed or enriched with these on-growing or similar diets are required to determine whether the survival and growth rates of rock lobster larvae can be improved. Although success in rearing *J. edwardsii* phyllosomata to pueruli has been limited (Kittaka, 1994a), we propose that the use of *Artemia* grown to 5 d on an OWL–oil or a similar LC-PUFA rich diet in an alternative format will be beneficial for the future of *J. edwardsii* aquaculture.

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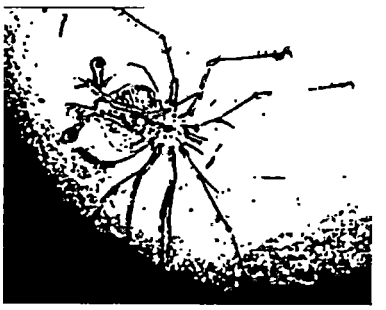
Chapter Three



Growth and lipid composition of phyllosomata of the southern rock lobster, *Jasus edwardsii*, fed enriched *Artemia*

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Abstract

Newly-hatched phyllosoma larvae of *Jasus edwardsii* were on-grown to stage V. Using triacylglycerol-rich marine oil nutrient sources and microalgae, *Artemia* were enriched with the major polyunsaturated fatty acids (PUFA) to ratios similar to that of wild-caught phyllosomata. *Artemia* enriched by different methods were fed to cultured phyllosomata. At each stage animals were counted, measured and sampled for lipid analyses. Survival was highest from stages II–III (62–86%), with mean total survival at 3–12%. From stages I–V larvae increased in mass (0.2–2.2 mg) and total length (2.1–5.8 mm), and decreased in total lipid. The major lipid class in all phyllosomata was polar lipid, followed by sterol, with no triacylglycerol detected. The main fatty acids were 18:1(n-9)c, 18:2(n-6), 16:0, 18:0, eicosapentaenoic acid [EPA; 20:5(n-3)], 18:1(n-7)c, arachidonic acid [AA; 20:4(n-6)] and docosahexaenoic acid [DHA; 22:6(n-3)]. On-grown phyllosomata had levels of AA and EPA similar to that of wild phyllosomata, but contained markedly lower levels of DHA. Strategies for enhancement of DHA levels will be needed for culture of rock lobster phyllosomata.

KEY WORDS: *Artemia*, fatty acids, *Jasus edwardsii*, lipids, lobster, phyllosoma

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Introduction

The increasing global interest in culture of rock lobster demands successful and novel culture protocols that are specific to the complex life cycle. For commercially efficient culture from egg to marketable size, determination of the requirements of the planktonic larval phase is paramount. Culture of phyllosomata to pueruli has been

achieved (Kittaka & Booth, 2000; Phillips & Liddy, 2003), although in limited numbers unsuitable for commercial scale. Even for the now commercially viable prawn aquaculture industry, it has taken considerable time to address larval rearing (Wickins, 1972; Kanazawa *et al.*, 1985), with research continuing (Abdel-Rahman, 1996; D'Souza *et al.*, 2002). As it is recognized that low survival is a current difficulty with rock lobster larviculture, research addressing important components of phyllosomata culture is vital. These include aquaria design (Greve, 1968; Illingworth *et al.*, 1997; Kittaka & Booth, 2000; Ritar, 2001), reducing microbial loading (Igarashi *et al.*, 1990; Diggles *et al.*, 2000), feeding capabilities (Batham, 1967; Kittaka, 1994a; Johnston & Ritar, 2001; Nelson *et al.*, 2002a; Cox & Johnston, 2003c) and nutrition.

Provision of appropriate nutrition requires clarification of essential dietary nutrients. Although information regarding roles of protein, carbohydrates, vitamins and inorganic elements are necessary, much less is know about lipid nutrition (Sargent *et al.*, 2002), of which the correct levels are fundamental (Conklin *et al.*, 1991; Kanazawa & Koshio, 1994; Saski *et al.*, 1996; Glencross *et al.*, 1998). Culture of phyllosomata by feeding *Artemia* and/or mussel tissue has been moderately effective (Kittaka, 1997b; Matsuda & Yamakawa, 1997; Tong *et al.*, 1997; Moss *et al.*, 1999). However, the lack of diets that contain lipid class and fatty acid profiles appropriate to phyllosomata has been a major constraint to the successful culture of palinurid lobsters. Although knowledge of natural food or prey items of phyllosomata is limited (Shojima, 1963; Thomas, 1963; Phleger *et al.*, 2001), the recent comprehensive examination of lipids and fatty acid profiles in wild phyllosomata (Phleger *et al.*, 2001) and potential prey items (Nichols *et al.*, 2001) have provided insight into lipid nutritional requirements. Subsequent applicable work was performed enriching *Artemia* with essential polyunsaturated fatty acids (PUFA) designed to mimic the composition of wild larvae (Phleger *et al.*, 2001; Nelson *et al.*, 2002b; Smith *et al.*, 2002a). Clarification of lipid nutrition in rock lobster phyllosoma larvae is a critical factor to achieve successful aquaculture; therefore the present study was performed to assess the effects of providing a PUFA-enriched *Artemia* diet on survival, growth and composition of *Jasus edwardsii* phyllosomata.

Methods

Artemia enrichment

Decapsulated *Artemia* cysts (Great Salt Lake Prime Gold, INVE Aquaculture, Baasrode, Belgium) were hatched at 28 ± 1 °C in 50-L white fiberglass cones that contained 0.2-μm filtered brackish water (27 ± 1 g L^{-1}), with vigorous aeration and a 150-W light suspended 0.5 m above the water. After 24 h, *Artemia* nauplii were removed from the hatching cones, rinsed in freshwater for 2 min and transferred to 1000-L tanks containing filtered seawater (0.2 μm, 34 ± 1 g L^{-1}, 27 ± 1 °C). *Artemia* were fed twice daily with a rice pollard–soy flour–wheat flour brine shrimp diet (Eyre Peninsula Aquafeeds, South Australia, Australia) at a rate that maintained a Secchi depth of 25–30 cm. Environmental conditions remained stable for the duration of the on-growing period; salinity (35.7 ± 0.2 g L^{-1}), pH (8.3 ± 0.0), dissolved oxygen (7–7.2 mg L^{-1}) and temperature (26.9 ± 0.1 °C). After 5 days, and directly prior to enrichment, 80,000 *Artemia* (1.5 ± 0.2 mm total length) were removed from the on-growing container, rinsed in freshwater for 2 min and transferred to 50-L white fiberglass cones containing 10 L of filtered seawater to achieve a density of 4 mL^{-1}.

Five-day old juvenile *Artemia* were enriched for 6 hrs with 0.6 g L^{-1} of six nutrients sources: [1] OWL–oils: a mix of oat bran–wheat germ–lecithin (OWL, 50:6:4) with the marine oils Omega pure (Omega Protein, Inc., Refined Oils, Virginia, USA) and Sun-TGA40S (Suntory Limited, Osaka, Japan) (4.5:1, 40% by mass), [2] OWL–oils–cholesterol (2% by mass), [3] DHA-Selco (INVE Aquaculture, Baasrode, Belgium), [4] the microalga *Chaetoceros muelleri*, [5] A1-Selco (INVE Aquaculture, Baasrode, Belgium) and [6] the microalga *Isochrysis galbana* (T ISO). The oat bran-based diets were prepared daily by homogenizing ingredients suspended in 500 mL of seawater in a household blender and sieving through a 63-μm screen. The Omega pure and Sun-TGA40S products were triacylglycerol (TAG) marine oils. *C. muelleri* and *I. galbana* were cultured and the cell density was measured daily as described in Wilkinson (2000).

Experimental aquaria

One thousand newly-hatched phyllosomata $aquarium^{-1}$ were on-grown to stage V in 10-L circular flow-through aquaria (Ritar, 2001). Each of six dietary treatments were duplicated and contained enriched *Artemia* (as described previously) with the follow-

ing protocol: [1] OWL–oils, [2] OWL–oils–cholesterol, [3] DHA-Selco–*C. muelleri* (1:2 v/v), [4] OWL–oils followed by DHA-Selco–*C. muelleri* at stage III (termed Switch diet), [5] A1-Selco and [6] *I. galbana*. Phyllosomata were fed the enriched *Artemia* in the evening, and aquaria were equipped with small screens (250-μm) to retain *Artemia*. In the morning, the small screens were replaced by large screens (1500-μm) to allow excess *Artemia* and debris to be flushed from the tanks. Extraneous debris and any mortalities were siphoned daily. After each molt, all animals in each aquarium were counted and the total length, carapace length and carapace width of 10 phyllosomata aquarium^{-1} were measured utilizing a dissecting microscope, digital camera and Scion Image Beta 4.0.2 software (Scion Corporation, Frederick, Maryland, USA).

Lipid extraction

Samples taken for lipid analyses consisted of: 1500 newly-hatched phyllosoma larvae (in duplicate from 3 broodstock), and from each aquarium 100 stage I, 50 stage II, 35 stage III, 20 stage IV and 10 stage V; all midstage. Nutrient sources, *Artemia* and phyllosomata samples were filtered through 4.7-cm Whatman glass filters (GF/F) and rinsed with 0.5-M ammonium formate. The majority of treatment samples were analyzed in duplicate; the level of replication for analyses is provided in the footnotes of each table. Samples were lyophilized overnight and quantitatively extracted overnight at 18 °C using a modified Bligh & Dyer (1959) one-phase methanol–chloroform–water extraction (2:1:0.8 v/v/v). The phases were separated by the addition of chloroform–water (final solvent ratio, 1:1:0.9 v/v/v methanol–chloroform–water). The total solvent extract (TSE) was concentrated using rotary evaporation at 40 °C.

Lipid classes

An aliquot of each TSE was analyzed using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to quantify individual lipid classes (Ackman, 1981; Volkman & Nichols, 1991). Samples were applied in duplicate to silica gel SIII chromarods (5-μm particle size) using 1-μL micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The primary solvent system used for the lipid separation was hexane–diethyl ether–acetic acid (60:17:0.1), a mobile phase resolving non-polar

compounds such as wax ester (WE), TAG, free fatty acids (FFA) and sterols (ST). A second non-polar solvent system consisting of hexane–diethyl ether (96:4) was also used to resolve hydrocarbons, WE, TAG and diacylglyceryl ether (DAGE). After development, the chromarods were oven dried and analyzed immediately to minimize absorption of atmospheric contaminants. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, TAG (derived from fish oil), WE (derived from orange roughy oil) and DAGE (derived from shark liver oil); 0.1–10 μg range]. Peaks were quantified on an IBM compatible computer using DAPA Scientific software (Kalamunda, Western Australia, Australia). TLC-FID results are generally reproducible to ±5–10% of individual class abundance (Volkman & Nichols, 1991).

Fatty acids

An aliquot of the total lipid was *trans*-methylated to produce fatty acid methyl esters (FAME) using methanol–chloroform–conc. hydrochloric acid (10:1:1, 80 °C, 2 hr). FAME were extracted into hexane–chloroform (4:1, 3 × 1.5 mL) and treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA 50 μl, 60 °C, overnight) to convert ST and alcohols to their corresponding TMSi ethers.

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC (Avondale, Pennsylvania, USA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of methyl nonodecanoate and methyl tricosanoate internal standards, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150 °C at 30 °C min^{-1}, then to 250 °C at 2 °C min^{-1}, and finally to 300 °C at 5 °C min^{-1}. Peaks were quantified with Waters Millennium software (Milford, Massachusetts, USA). Individual components were identified by using mass spectral data and comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, Texas, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described previously.

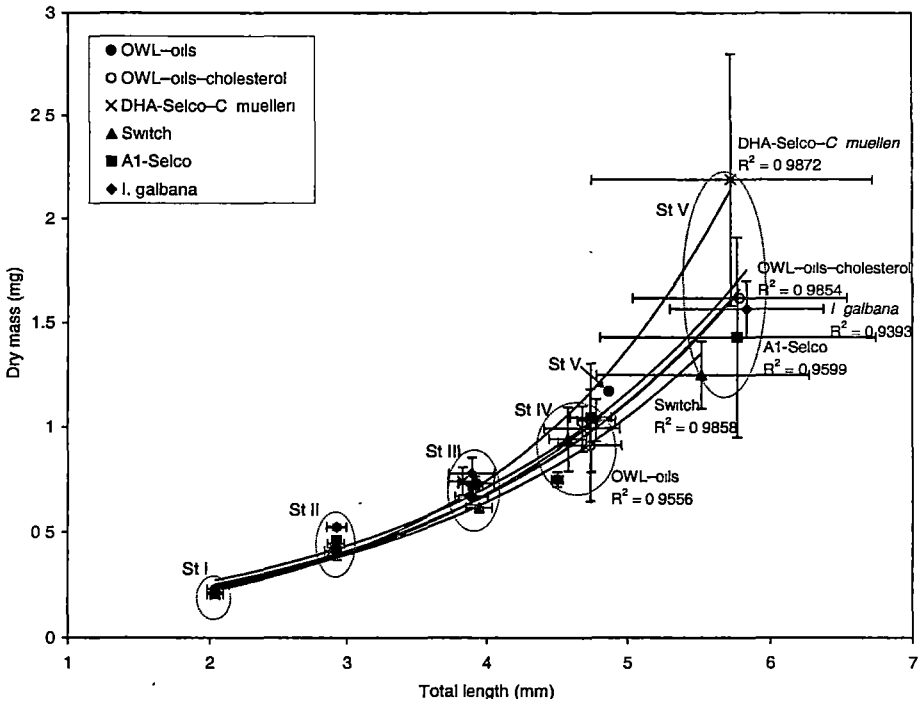

Figure 1 Dry mass as a function of total length of *Jasus edwardsii* phyllosomata from stages I–V on six dietary treatments (mean ± SE). Switch treatment defined as OWL–oils followed by DHA-Selco–*C. muelleri* at stage III.

Statistical analyses

Fatty acid profiles (mg g^{-1} dry mass) of individual samples (nutrient sources, *Artemia* and phyllosomata) were compared in two dimensions, using the Kruskal Loss Function, by Pearson's correlation coefficient and non-metric multidimensional scaling (MDS). All multivariate analyses were conducted using SYSTAT 10 (SYSTAT, Inc., Evanston, Illinois, USA).

Results

Feeding

Due to the feeding behavior of *Artemia* and phyllosoma, it was not possible to quantify food consumption and therefore calculate ingestion rates and feed conversion efficiency. However, daily observations indicated that both *Artemia* and phyllosomata were feeding. *Artemia* had full digestive tracts and faecal trails. While in the aquaria the phyllosomata were observed feeding on whole or partial *Artemia* grasped in their periopods and/or maxillipeds. These observations, combined with the confirmed incorporation of dietary-derived FA (see below), provide good evidence that the *Artemia* consumed their nutrient sources and the phyllosomata consumed the diets (*Artemia*).

Table 1 Percentage survival of phyllosoma from enrichment trial (mean ± SE)

	Stage				
Diet treatments	I–II	II–III	III–IV	IV–V	Total
OWL–oils	56.9 ± 0.6	61.6 ± 27.3	25.8 ± 9.5	35.2 ± 0.5	2.7 ± 0.3
OWL oils cholesterol	50.1 ± 10.8	76.6 ± 2.7	48.2 ± 34.7	37.3 ± 19.1	9.6 ± 8.3
DHA-Selco–*C. muelleri*	63.4 ± 10.1	85.9 ± 2.9	25.8 ± 14.0	20.9 ± 18.1	3.8 ± 3.6
Switch[1]	54.1 ± 9.3	65.3 ± 6.0	33.1 ± 2.6	46.5 ± 28.5	4.5 ± 1.6
A1-Selco	59.1 ± 5.5	85.7 ± 2.5	59.5 ± 16.7	35.1 ± 19.5	11.6 ± 8.0
I. galbana	49.4 ± 0.7	64.1 ± 4.0	21.3 ± 1.4	51.4 ± 29.5	3.2 ± 1.6

n = 2; [1]OWL–oils followed by DHA-Selco–*C. muelleri* at stage III.

Morphometrics

Phyllosomata in all treatments increased in total length (2.1–5.8 mm) and mass per individual (0.2–2.2 mg dry mass) from stages I–V (Fig. 1). The increases in total length and mass in phyllosomata are similar among all diet treatments, with good fit of exponential trend lines ($R^2 \geq 0.94$; Fig. 1). Variation at early developmental stages is minimal, with larger variations occurring at stage V. A significant among-treatment difference is seen for stage V, OWL–oils-fed phyllosomata, which are inferior to all other treatments in both length and mass.

Percentage survival varied between each stage, and was highest from stages II–III (62–86%; Table 1). Total survival for all treatments were OWL–oils (2.7%), OWL–oils–cholesterol (9.6%), DHA-Selco–*C. muelleri* (3.8%), Switch (4.5%), A1-Selco (11.6%) and *I. galbana* (3.2%). There were no differences in intermolt period for phyllosomata among treatments. Intermolt periods were 12, 10, 11 and 14 days for stages I–II, II–III, III–IV, IV–V, respectively.

Lipid content and classes

In *Artemia*, lipid content was similar among those fed the laboratory-prepared enrichment diets (i.e., nutrient sources) (112–120 mg·g^{-1} dry mass) and slightly lower for algal-enriched *Artemia* (88–90 mg·g^{-1}; Table 2). Polar lipid (PL) was the major lipid class (60–89% of total lipid; Table 2), followed by TAG. The level of TAG was much lower in algal-enriched *Artemia* (2–3%) compared to that of other nutrient sources (19–34%). ST (3–5%), free fatty acid (FFA; 3–4%) and WE (0.2–3%) were minor components. No diacylglycerol (DG) was detected in *Artemia*. The nutrient sources all contained >89% TAG (Table 2).

In phyllosomata, although lipid per individual generally increased from stages I–V (20–38 to 40–115 μg), the absolute lipid content decreased (97–180 to 34–76 mg

Table 2 Percentage lipid class composition of nutrient sources and *Artemia* from enrichment trial (mean ± SD)

	Wax ester	Triacylglycerol	Free fatty acid	Sterol	Polar lipid	Lipid as mg g^{-1} dry mass
Nutrient sources						
OWL–oils	0.6 ± 0.4	93.5 ± 0.8	0.8 ± 0.5	1.4 ± 0.7	3.7 ± 1.0	575 ± 44
OWL–oils–cholesterol	0.6 ± 0.3	92.8 ± 0.1	0.8 ± 0.5	1.9 ± 0.1	3.9 ± 0.7	555 ± 15
DHA-Selco	–	93.2 ± 0.2	0.4 ± 0.3	0.5 ± 0.0	5.9 ± 0.1	827 ± 36
A1-Selco	–	88.7 ± 0.9	1.2 ± 0.0	0.6 ± 0.0	9.4 ± 0.9	932 ± 18
Artemia						
OWL–oils[1]	0.2 ± 0.1	34.1 ± 6.4	4.1 ± 1.8	3.3 ± 0.8	63.5 ± 8.8	120 ± 15
OWL–oils–cholesterol[1]	0.8 ± 0.7	20.3 ± 10.3	3.4 ± 1.2	4.1 ± 0.5	71.4 ± 11.1	117 ± 18
DHA-Selco	2.6 ± 0.1	31.3 ± 0.6	3.0 ± 0.1	3.2 ± 0.1	59.8 ± 0.5	116 ± 8
C. muelleri	1.5 ± 1.0	2.2 ± 2.1	3.3 ± 0.4	4.4 ± 0.1	88.6 ± 1.3	90 ± 4
A1-Selco	1.1 ± 0.8	18.9 ± 2.1	4.1 ± 0.3	3.9 ± 0.4	71.9 ± 2.0	112 ± 10
I. galbana	1.6 ± 0.8	2.9 ± 2.6	3.0 ± 0.1	5.0 ± 0.1	87.5 ± 1.6	88 ± 12

$n = 2$; [1]$n = 4$; (–), below detection; diacylglycerol below detection.

g^{-1} dry mass; Table 3). Newly-hatched phyllosomata in this study contained 87 $mg \cdot g^{-1}$ of lipid. PL comprised the major lipid class in all phyllosoma samples (83–95% of total lipid; Table 3), followed by ST (3–8%; mainly cholesterol), FFA (1–4%), WE (1–6%) and DG (1–2%; not detected in newly-hatched phyllosomata). No TAG was detected in any fed phyllosomata and newly-hatched phyllosomata contained only a minor amount (0.4%). By stage V, larvae from all treatments had slightly lower levels of PL than earlier stages (83–90% and 91–95%, respectively) and higher ST (6–7% and 3–4%, respectively; Table 3). On an absolute basis, the drop in PL was more apparent (88–162 to 30–65 mg g^{-1} dry mass), with minor changes revealed for ST.

Fatty acids

The fatty acids (FA) dominant in nutrient sources in decreasing order of proportional abundance of total FA were: palmitic acid (16:0; 17–20%), oleic acid [18:1(n-9)c; 14–16%], docosahexaenoic acid [DHA; 22:6(n-3); 8–21%], eicosapentaenoic acid [EPA; 20:5(n-3); 5–15%], linoleic acid [18:2(n-6); 5–9%], 16:1(n-7)c (4–9%;) and arachidonic acid [AA; 20:4(n-6); 1%; Table 4]. Notable features were high DHA in DHA-Selco (21%), and low AA (1%) and high EPA (15%) in A1-Selco.

The major FA in enriched *Artemia* were: 18:1(n-9)c (22–28%), 18:2(n-6) (12–21%), 16:0 (9–15%), *cis*-vaccenic acid [18:1(n-7)c; 6–10%] and stearic acid (18:0; 6–8%; Table 4). Essential PUFA were 2–4% AA, 5–9% EPA, with greater differences in DHA composition. *Artemia* enriched with DHA-Selco had slightly higher DHA; (10%), while algal-enriched *Artemia* had only minor amounts (0.2–0.9%; Table 4).

Table 3 Percentage lipid class composition of phyllosomata from enrichment trial (mean ± SD)

	Wax ester	Triacylglycerol	Free fatty acid	Diacylglycerol	Sterol	Polar lipid	Lipid as mg g^{-1} dry mass	Lipid indiv^{-1} dry mass (μg)
Diet treatments								
Stage								
Newly-hatched[1]	0.7 ± 0.1	0.4 ± 0.1	2.9 ± 1.6	–	8.0 ± 1.0	88.1 ± 2.7	87 ± 13	6 ± 1
OWL–oils								
I	0.9 ± 0.2	–	3.4 ± 1.4	0.6 ± 0.1	3.5 ± 0.1	91.7 ± 1.5	177 ± 52	38 ± 13
III	0.7 ± 0.1	–	2.9 ± 0.2	1.7 ± 0.1	3.5 ± 0.3	91.1 ± 0.1	83 ± 2	56 ± 6
V	2.4 ± 0.7	–	3.0 ± 2.2	1.9 ± 0.4	6.9 ± 2.6	85.8 ± 4.6	34 ± 12	40 ± 14
OWL–oils–cholesterol								
I	1.4 ± 0.4	–	3.3 ± 0.3	0.8 ± 0.1	3.2 ± 0.5	91.2 ± 0.2	156 ± 19	36 ± 1
III	0.6 ± 0.8	–	2.1 ± 0.7	1.8 ± 0.3	4.0 ± 1.3	91.5 ± 3.1	123 ± 48	90 ± 34
V	3.1 ± 2.4	–	1.0 ± 0.1	1.8 ± 0.1	5.4 ± 0.2	88.7 ± 2.6	65 ± 4	105 ± 7
DHA-Selco–*C. muelleri*								
I	1.3 ± 0.1	–	2.9 ± 0.6	0.5 ± 0.1	2.4 ± 0.2	92.9 ± 0.6	156 ± 65	32 ± 13
III	0.3 ± 0.4	–	1.5 ± 0.4	1.0 ± 0.1	2.5 ± 0.4	94.7 ± 1.4	119 ± 37	90 ± 38
V	1.3 ± 0.1	–	0.8 ± 0.0	1.7 ± 0.1	5.9 ± 0.3	90.3 ± 0.6	50 ± 19	100 ± 0
Switch[2]								
I	1.0 ± 0.6	–	2.9 ± 0.3	0.8 ± 0.2	2.8 ± 0.2	92.6 ± 1.3	128 ± 53	30 ± 14
III	0.5 ± 0.6	–	2.3 ± 0.9	1.5 ± 0.4	3.1 ± 0.4	92.6 ± 1.1	93 ± 1	57 ± 0
V	6.3 ± 8.2	–	1.8 ± 1.0	1.6 ± 0.4	7.3 ± 2.6	83.0 ± 11.4	56 ± 2	70 ± 11
A1-Selco								
I	1.0 ± 0.3	–	4.1 ± 0.6	0.9 ± 0.3	3.4 ± 0.5	90.6 ± 0.0	97 ± 39	20 ± 8
III	0.6 ± 0.0	–	2.0 ± 0.8	2.2 ± 0.7	4.1 ± 0.4	91.1 ± 0.4	131 ± 58	96 ± 51
V	4.9 ± 6.3	–	1.6 ± 1.1	1.9 ± 0.2	5.8 ± 0.0	85.8 ± 7.2	76 ± 18	115 ± 78
I. galbana								
I	1.4 ± 0.3	–	3.7 ± 0.5	0.7 ± 0.2	3.7 ± 0.8	90.5 ± 1.2	167 ± 20	36 ± 4
III	0.8 ± 0.2	–	2.3 ± 0.4	1.8 ± 0.6	3.8 ± 0.5	91.3 ± 0.8	94 ± 18	74 ± 24
V	0.9 ± 0.5	–	1.2 ± 0.7	2.3 ± 0.7	5.9 ± 1.4	89.7 ± 0.9	54 ± 6	86 ± 20

$n = 2$; [1]$n = 6$; [2]OWL–oils followed by DHA-Selco–*C. muelleri* at stage III; (–), below detection.

Table 4 Percentage fatty acid composition of nutrient sources and *Artemia* used in enrichment trial (mean ± SD)

	Nutrient sources				*Artemia*					
	OWL–oils	OWL–oils–cholesterol	DHA-Selco	A1-Selco	OWL–oils	OWL–oils–cholesterol	DHA-Selco	*C. muelleri*	A1-Selco	*I. galbana*
14:0	4.7 ± 1.0	4.7 ± 1.0	3.2 ± 0.0	6.5 ± 0.4	2.6 ± 0.3	1.9 ± 0.2	1.8 ± 0.1	1.0 ± 0.0	3.0 ± 0.4	1 4 ± 0.1
16:1(n-7)c	6.4 ± 1.4	6.3 ± 1.2	4.4 ± 0.3	8.9 ± 1.1	5.3 ± 0.7	4.5 ± 0.6	3.9 ± 0.0	5.0 ± 0.3	5.5 ± 0.2	3.7 ± 0.1
16:0	16.9 ± 1.5	17.7 ± 2.6	20.4 ± 0.0	16.7 ± 0.1	13.3 ± 0.8	11.5 ± 1.6	15.1 ± 0.6	8.5 ± 0.2	13.5 ± 0.5	9.0 ± 0.1
18:4(n-3)	2.7 ± 0.6	2.7 ± 0.6	0.9 ± 0.0	2.7 ± 0.0	1.7 ± 0.1	1.5 ± 0.2	1.0 ± 0.0	1.0 ± 0.0	1.5 ± 0.1	1.5 ± 0.2
18:2(n-6)/a18:0	9.3 ± 0.3	9.0 ± 0.3	5.6 ± 0.1	5.1 ± 0.2	16.1 ± 2.5	20.9 ± 8.7	11.7 ± 3.0	17.2 ± 2.3	12.3 ± 2.7	18.2 ± 1.9
18:1(n-9)c	13.7 ± 0.5	13.5 ± 0.1	15.5 ± 0.2	14.2 ± 0.9	22.6 ± 0.9	23.4 ± 2.7	22.4 ± 3.2	27.3 ± 1.6	23.1 ± 2.3	28.3 ± 2.1
18:1(n-7)c	2.3 ± 0.3	2.3 ± 0.2	2.6 ± 0.2	3.0 ± 0.1	5.7 ± 0.7	6.2 ± 1.4	6.4 ± 0.1	9.7 ± 1.6	7.4 ± 0.6	9.5 ± 1.4
18:0	3.8 ± 0.3	3.8 ± 0.4	6.1 ± 0.2	3.5 ± 0.1	6.1 ± 0.8	6.2 ± 1.4	7.9 ± 0.1	8.4 ± 1.1	7.1 ± 0.5	8.3 ± 0.9
20:4(n-6)	7.5 ± 0.1	7.2 ± 0.0	1.9 ± 0.1	1.0 ± 0.1	4.1 ± 0.2	3.5 ± 0.5	2.3 ± 0.3	2.7 ± 0.2	1.8 ± 0.1	2.5 ± 0.4
20:5(n-3)	8.7 ± 1.4	8.6 ± 1.5	5.0 ± 0.5	15.0 ± 0.5	5.7 ± 0.3	5.6 ± 0.5	5.5 ± 0.3	6.7 ± 0.3	8.6 ± 0.7	5.0 ± 0.0
22:6(n-3)	8.2 ± 3.6	8.2 ± 3.5	21.1 ± 0.1	8.0 ± 0 3	3.2 ± 0.8	2.3 ± 0.5	9.7 ± 4 0	0.2 ± 0.0	3.0 ± 0.7	0.9 ± 0.2
Other	15.9	15.9	13.3	15.4	13.6	12.4	12 3	12.2	13 2	11.6
Sum SFA	29.6 ± 2.6	30.6 ± 4.0	34.1 ± 0.3	31.3 ± 0.6	26 6 ± 2.0	24.2 ± 4.1	29.9 ± 1.1	23.5 ± 1.7	28.9 ± 1.8	24.2 ± 1.4
Sum MUFA	39.8 ± 0.7	39.3 ± 0.1	39.7 ± 0.3	42.1 ± 2.8	55.5 ± 1.6	55.7 ± 6.0	52.5 ± 6.1	62.8 ± 2.9	55.9 ± 4.1	63.9 ± 3.9
Sum PUFA	42.2 ± 3.2	41.4 ± 4.0	38.7 ± 0.3	37.6 ± 1.2	35.0 ± 2.7	37.6 ± 8.5	33.6 ± 2.0	31.6 ± 2.1	31.1 ± 0.7	31.3 ± 0.9
Sum (n-3)	21.8 ± 4.3	21.7 ± 4.5	28.4 ± 0.5	28.4 ± 0.9	11.7 ± 0.6	10.2 ± 1.3	16.8 ± 4.5	8.1 ± 0.3	14.2 ± 1.8	7.6 ± 0 5
Sum (n-6)	18.2 ± 0.5	17.6 ± 0.1	9.7 ± 0.1	6.7 ± 0.2	21.4 ± 2.5	25.7 ± 8.4	15.8 ± 2.3	21.4 ± 1.8	15.1 ± 2.4	22.1 ± 1.3
Ratio (n-3)/(n-6)	1.2	1.2	2.9	4.2	0.5	0.4	1.1	0.4	0.9	0.3
Ratio EPA/AA	1.2	1.2	2.7	15.8	1.4	1.6	2.4	2.4	4.7	2.0
Ratio DHA/EPA	0.9	0.9	4.2	0.5	0.6	0.4	1.8	0.0	0.4	0.2

n = 2; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Other includes components present at <2%: i15:0, a15:0, 15:0, i16:0, C_{16} PUFA, 16:1(n-9)c, 16:1(n-7)t/16:2, 16:1(n-5)c, 16:0 Falde (fatty aldehyde), i17:0, a17:0, 17:1, 17:0, 18:3(n-6), i18:0, 18:1(n-7)t, 18:1(n-5)c, 18:0 Falde, i19:0, 19:1, 20.3(n-6), 20:4(n-3), 20:2(n-6), 20:1(n-11)c, 20:1(n-7)c, 20:0, C_{21} PUFA, 21:0, 22:5(n-6), 22:4(n-6), 22:5(n-3), 22:1(n-11), 22:1(n-9), 22:1(n-7), 22:0, 24:1, 24:0.

Table 5 Percentage fatty acid composition of phyllosoma from enrichment trial (mean ± SD)

Diet treatments		OWL–oils			OWL–oils–cholesterol			DHA-Selco–*C. muelleri*		
Stage	New hatch[1]	I	III	V	I	III	V	I	III	V
16:1(n-7)c	2.9 ± 0.2	2.4 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	2.2 ± 0.0	1.7 ± 0.1	2.0 ± 0.2	2.1 ± 0.0	2.2 ± 0.0	2.8 ± 0.6
16:0	14.4 ± 0.5	11.3 ± 0.1	11.6 ± 0.4	12.0 ± 1.2	11.3 ± 0.1	11.3 ± 0.4	11.8 ± 0.8	11.1 ± 0.2	11.0 ± 0.1	11.9 ± 0.1
18:2(n-6)/a18:0	1.3 ± 0.2	18.2 ± 0.0	20.3 ± 0.4	13.7 ± 2.2	18.7 ± 0.1	21.1 ± 0.9	16.1 ± 0.0	16.7 ± 0.1	19.7 ± 0.5	14.0 ± 2.6
18:1(n-9)c	14.6 ± 1.8	24.7 ± 0.2	24.6 ± 0.8	19.6 ± 2.5	25.5 ± 0.3	25.8 ± 0.7	22.3 ± 0.8	25.7 ± 0.1	27.2 ± 0.7	23.6 ± 0.3
18:1(n-7)c	4.3 ± 0.2	5.9 ± 0.1	5.7 ± 0.0	5.8 ± 0.7	6.0 ± 0.1	5.7 ± 0.0	6.4 ± 0.5	6.0 ± 0.4	6.0 ± 0.1	5.2 ± 0.6
18:0	7.2 ± 0.2	8.0 ± 0.6	9.1 ± 0.6	9.4 ± 0.8	7.9 ± 0.0	8.7 ± 0.4	9.3 ± 0.2	9.5 ± 1.8	8.4 ± 0.1	8.3 ± 2.3
20:4(n-6)	12.8 ± 0.7	5.6 ± 0.3	4.6 ± 0.2	5.3 ± 0.8	5.3 ± 0.3	4.2 ± 0.2	5.1 ± 0.2	4.3 ± 0.4	2.8 ± 0.3	2.9 ± 0.9
20:5(n-3)	15.5 ± 0.8	9.0 ± 0.1	8.0 ± 0.0	9.3 ± 0.8	9.0 ± 0.1	7.9 ± 0.2	9.2 ± 0.7	9.7 ± 0.7	8.4 ± 0.4	8.6 ± 0.9
20:2(n-6)	2.3 ± 0.1	1.0 ± 1.4	2.7 ± 0.0	2.5 ± 0.2	1.9 ± 0.0	2.8 ± 0.1	2.8 ± 0.1	1.8 ± 0.0	2.4 ± 0.1	2.0 ± 1.0
20:1(n-9)c	2.1 ± 0.1	0.7 ± 1.0	1.4 ± 0.0	1.1 ± 0.2	1.3 ± 0.0	1.4 ± 0.1	0.7 ± 0.9	1.2 ± 0.1	0.7 ± 0.9	0.6 ± 0.9
22:6(n-3)	9.6 ± 0.5	3.1 ± 0.3	2.5 ± 0.2	3.4 ± 0.7	3.0 ± 0.0	2.0 ± 0.3	1.7 ± 2.2	4.0 ± 0.2	2.9 ± 0.2	4.2 ± 0.5
Other	12.9	10.0	9.1	16.0	7.9	7.5	12.6	7.9	8.3	14.8
Sum SFA	27.1 ± 0.3	23.3 ± 0.1	24.5 ± 1.2	33.4 ± 9.2	22.9 ± 0.0	23.6 ± 1.0	24.7 ± 1.8	24.7 ± 2.1	23.1 ± 0.3	25.8 ± 1.0
Sum MUFA	37.2 ± 2.9	56.1 ± 1.3	54.5 ± 1.8	44.6 ± 5.5	56.5 ± 0.5	56.7 ± 1.4	52.9 ± 2.3	56.9 ± 0.1	60.2 ± 1.4	56.8 ± 5.3
Sum PUFA	46.0 ± 0.7	39.2 ± 1.7	39.5 ± 0.0	35.6 ± 5.2	39.9 ± 0.3	39.4 ± 0.3	38.2 ± 0.9	37.9 ± 1.5	37.4 ± 0.4	34.2 ± 3.8
Sum (n-3)	26.7 ± 0.7	13.4 ± 0.1	11.2 ± 0.2	13.2 ± 2.0	13.0 ± 0.0	10.6 ± 0.3	11.6 ± 3.0	14.3 ± 0.9	11.8 ± 0.6	13.8 ± 0.0
Sum (n-6)	18.6 ± 0.9	25.4 ± 1.5	27.9 ± 0.2	21.9 ± 3.2	26.4 ± 0.2	28.4 ± 0.6	26.0 ± 2.2	23.2 ± 0.6	25.2 ± 0.2	19.9 ± 3.8
Ratio (n-3)/(n-6)	1.4	0.5	0.4	0.6	0.5	0.4	0.4	0.6	0.5	0.7
Ratio EPA/AA	1.2	1.6	1.7	1.7	1.7	1.9	1.8	2.3	3.0	2.9
Ratio DHA/EPA	0.6	0.3	0.3	0.4	0.3	0.2	0.2	0.4	0.3	0.5
Total μg indiv^{-1} FA	4.5 ± 0.5	11.9 ± 2.4	31.3 ± 5.7	19.4 ± 2.3	11.9 ± 1.1	41.7 ± 5.9	47.5 ± 15.9	7.1 ± 6.0	44.6 ± 4.5	41.7 ± 37.9

$n = 2$; [1]$n = 6$; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; (–), below detection; Other includes components present at <2%: 14:0, i15:0, a15:0, 15:0, i16:0, C_{16} PUFA, 16:1(n-9)c, 16:1(n-7)t/16:2, 16:1(n-5)c, 16:0 Falde (fatty aldehyde), i17:0, a17:0, 17:1, 17:0, 18:3(n-6), 18:4(n-3) i18:0, 18:1(n-7)t, 18:1(n-5)c, 18:0 Falde, i19:0, 19:1, 20:3(n-6), 20:4(n-3), 20:1(n-11)c, 20:1(n-7)c, 20:0, C_{21} PUFA, 21:0, 22:4(n-6), 22:5(n-3), 22:5(n-6), 22:1(n-11), 22:1(n-9), 22:1(n-7), 22:0, 24:1, 24:0.

Table 5 Percentage fatty acid composition of phyllosoma from enrichment trial (mean ± SD) (continued)

Diet treatments	Switch[1]			A1-Selco			*I. galbana*		
Stage	I	III	V	I	III	V	I	III	V
16:1(n-7)c	2.4 ± 0.1	1.7 ± 0.1	2.3 ± 0.6	2.3 ± 0.0	2.1 ± 0.0	2.2 ± 0.5	1.8 ± 0.0	1.6 ± 0.0	2.0 ± 0.1
16:0	11.3 ± 0.2	10.9 ± 1.1	13.3 ± 1.1	11.3 ± 0.3	11.1 ± 0.2	11.9 ± 0.7	11.6 ± 0.3	11.2 ± 0.2	11.9 ± 0.6
18:2(n-6)/a18:0	18.6 ± 0.3	19.9 ± 1.7	14.5 ± 2.6	16.5 ± 0.4	18.5 ± 0.4	15.6 ± 0.4	19.6 ± 0.8	21.3 ± 0.1	17.6 ± 0.8
18:1(n-9)c	24.9 ± 0.4	24.2 ± 2.1	22.3 ± 3.3	25.6 ± 0.6	26.6 ± 0.6	23.1 ± 1.6	26.7 ± 0.7	26.9 ± 0.2	23.7 ± 0.4
18:1(n-7)c	5.9 ± 0.1	5.4 ± 0.5	3.6 ± 3.3	6.2 ± 0.3	6.2 ± 0.1	6.8 ± 0.7	5.9 ± 0.2	5.7 ± 0.0	6.8 ± 0.1
18:0	7.8 ± 0.0	8.2 ± 0.9	10.2 ± 0.0	8.0 ± 0.1	8.5 ± 0.1	9.5 ± 0.2	8.3 ± 0.2	9.0 ± 0.0	9.9 ± 0.0
20:4(n-6)	5.2 ± 0.2	4.1 ± 0.3	4.5 ± 0.5	3.7 ± 0.3	2.5 ± 0.1	4.4 ± 2.3	4.1 ± 0.7	3.0 ± 0.2	3.7 ± 0.1
20:5(n-3)	9.0 ± 0.0	7.5 ± 0.6	12.0 ± 2.9	11.2 ± 0.3	10.5 ± 0.0	10.7 ± 0.5	8.1 ± 0.7	7.4 ± 0.2	8.3 ± 0.2
20:2(n-6)	1.9 ± 0.1	2.6 ± 0.2	2.7 ± 0.0	0.9 ± 1.3	2.5 ± 0.0	2.6 ± 0.3	1.9 ± 0.0	2.7 ± 0.1	2.8 ± 0.0
20:1(n-9)c	1.3 ± 0.1	–	0.6 ± 0.9	0.6 ± 0.8	0.7 ± 0.9	0.6 ± 0.9	1.2 ± 0.0	0.7 ± 1.0	0.6 ± 0.9
22:6(n-3)	3.3 ± 0.3	2.1 ± 0.1	4.4 ± 0.4	3.8 ± 0.6	2.5 ± 0.4	3.3 ± 0.8	2.8 ± 0.3	1.8 ± 0.1	2.2 ± 0.3
Other	8.3	13.4	9.6	9.8	8.3	9.2	8.2	8.7	10.5
Sum SFA	23.0 ± 0.5	27.8 ± 5.6	28.5 ± 1.4	23.2 ± 0.6	23.2 ± 0.4	25.6 ± 1.2	23.8 ± 0.2	24.1 ± 0.1	26.3 ± 0.3
Sum MUFA	55.6 ± 0.6	53.3 ± 4.3	50.4 ± 8.0	58.1 ± 0.6	58.7 ± 1.1	51.8 ± 4.4	58.4 ± 1.3	59.0 ± 0.7	53.5 ± 0.6
Sum PUFA	40.2 ± 0.2	37.5 ± 2.9	39.7 ± 0.1	38.0 ± 1.6	38.1 ± 0.1	38.6 ± 2.1	38.3 ± 0.8	37.9 ± 0.3	37.2 ± 0.3
Sum (n-3)	13.5 ± 0.4	10.3 ± 0.7	17.2 ± 2.3	16.1 ± 0.4	14.0 ± 0.4	15.1 ± 0.1	11.8 ± 0.9	10.0 ± 0.3	11.8 ± 0.4
Sum (n-6)	26.2 ± 0.6	26.9 ± 2.2	22.0 ± 2.3	21.5 ± 2.0	23.7 ± 0.3	23.0 ± 2.3	26.0 ± 0.0	27.3 ± 0.0	24.6 ± 0.6
Ratio (n-3)/(n-6)	0.5	0.4	0.8	0.7	0.6	0.7	0.5	0.4	0.5
Ratio EPA/AA	1.7	1.8	2.7	3.0	4.2	2.4	2.0	2.5	2.2
Ratio DHA/EPA	0.4	0.3	0.4	0.3	0.2	0.3	0.3	0.2	0.3
Total μg indiv^{-1} FA	11.9 ± 0.7	37.2 ± 4.8	36.0 ± 29.9	12.0 ± 1.9	42.8 ± 8.7	88.8 ± 19.0	11.4 ± 0.9	45.2 ± 11.8	57.3 ± 19.1

[1]OWL–oils followed by DHA-Selco–*C. muelleri* at stage III.

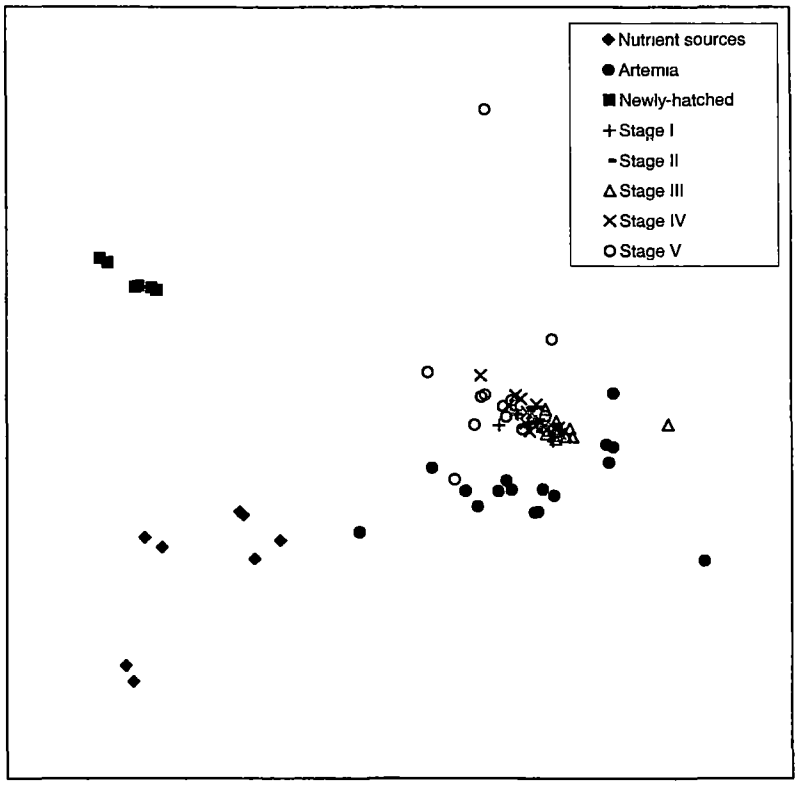

Figure 2 Scatterplot of multidimensional scaling (MDS) comparing nutrient sources, *Artemia* and *Jasus edwardsii* phyllosomata from feeding trial using a suite of fatty acids (individual samples; mg g^{-1} dry mass); Stress = 0.07311, Proportion of variance = 0.98918. Axis scales are arbitrary in non-metric MDS and are therefore omitted.

In phyllosomata, the major FA were similar to those found in the enriched *Artemia* and in decreasing order of abundance were: 18:1(n-9)c (20–27% of total FA), 18:2(n-6) (14–21%), 16:0 (11–13%), 18:0 (8–10%), EPA (8–9%), 18:1(n-7)c (6%), AA (3–6%) and DHA (2–4%; Table 5). In spite of the different levels of the essential PUFA in the enriched *Artemia* fed to the larvae, no marked differences were found among phyllosoma stages or diet treatments. Newly-hatched phyllosomata contained comparatively higher proportions of AA (13%), EPA (15%) and DHA (10%), with less 18:1(n-9)c (15%; Table 5).

The multidimensional scaling (MDS) of fatty acid profiles resulted in separation in the scatterplot among newly-hatched phyllosomata, nutrient sources and *Artemia*. The plot additionally highlighted separation among nutrient sources and *Artemia*. Early stages of phyllosomata were tightly clustered and separated at later stages (Fig. 2).

Discussion

Significance of polar lipid

Intermolt period and growth between stages for phyllosomata in this feeding trial compared well to those reported previously for this species (Tong *et al.*, 1997; Moss *et al.*, 1999; Ritar *et al.*, 2002; Ritar *et al.*, 2003a). A distinct change at stage III was apparent for survival of phyllosomata. Survival was high between stages I–II and II–III for all dietary treatments, and after stage III decreases proportionately with decrease in lipid. That is, survival decreases as total lipid in the larvae falls below 100 mg g^{-1} dry mass. Larvae from the present study exhibit an overall decrease in total lipid (97–180 to 34–76 mg g^{-1} dry mass) from stages I–V with the content of the cultured stage V phyllosomata being markedly less than that of wild *J. edwardsii* larvae (250 mg g^{-1} dry mass) (Phleger *et al.*, 2001). PL was the predominant lipid class in all phyllosomata (≥84% of total lipid). Like the cultured phyllosomata, wild *J. edwardsii* phyllosomata were extremely low in TAG (below detection–0.3% of total lipid), with PL as the major lipid class (≥83% of total lipid) (Phleger *et al.*, 2001). After metamorphosing from phyllosomata to non-feeding pueruli, there is a marked depletion of PL (Jeffs *et al.*, 2001a; Jeffs *et al.*, 2002). This suggests that the non-feeding pueruli use PL as an energy source; for other marine species energy is traditionally derived from TAG. The potential prey items of *J. edwardsii* also consist of high levels PL (≥88%), with TAG generally found at lower levels (Nichols *et al.*, 2001). Although most enriched *Artemia* diets contained appreciable amounts of TAG (19–34%), no TAG were detected in phyllosomata. The newly-hatched phyllosomata contained high levels of PL (percent basis), the relative proportion of which remained constant through the stages. This suggests a disproportional increase in membrane lipid only. This indicates that phyllosomata were not storing lipid during our feeding trial. Although the very low TAG suggests that the cultured animals were starved or not being properly fed, wild phyllosomata had similarly low TAG. Furthermore, the cultured larvae were clearly consuming *Artemia*. Not only were they observed grasping whole or partial *Artemia*, but phyllosomata would not molt if they had not been feeding (Abrunhosa & Kittaka, 1997a). Since the phyllosomata may not be storing lipid, we suggest they may not efficiently metabolize and incorporate the TAG derived from enriched *Artemia*. Also, the presence of DG in phylloso-

mata, and not in *Artemia*, may indicate a secondary energy reserve role in larvae, as suggested for wild phyllosomata (Phleger *et al.*, 2001) and pueruli (Jeffs *et al.*, 2001a; Jeffs *et al.*, 2002). Alternatively, DG may simply reflect lipase activity on PL. Our results suggest that diets high in TAG are not appropriate for rock lobster phyllosomata.

Essential polyunsaturated fatty acids

In the MDS scatterplot of FA profiles (mg g^{-1} dry mass), statistical analyses exhibit clear separation among newly-hatched phyllosomata, nutrient sources and *Artemia*, and trends for individual FA indicate that among phyllosoma stages and dietary treatments, essential PUFA profiles did not differ considerably. The relative levels of AA (3–6%) and EPA (8–9%) were comparable to those of wild-caught phyllosomata (2–3% AA; 7–9% EPA), but the levels of DHA were markedly lower (2–4%) than wild larvae (16–17% DHA) (Phleger *et al.*, 2001). Values of AA and EPA in the cultured phyllosomata are similar to their enriched *Artemia* diets (2–4% AA; 5–9% EPA). Although the DHA levels achieved in phyllosomata fed *Artemia* enriched with OWL–oils, OWL–oils–cholesterol, DHA-Selco–*C. muelleri* [a 1:2 ratio of DHA-Selco (9.7%) and *C. muelleri* (0.2%) produces 3.4% DHA] and A1-Selco were similar to levels in the respective *Artemia* diets (2–4% DHA), the percent of DHA was dissimilar in *I. galbana*-enriched *Artemia* (0.9%). *I. galbana* ineffectively maintains high DHA in *Artemia* (Olsen *et al.*, 2000). The higher value of DHA in *I. galbana* treatment phyllosomata (2–3%) compared with *I. galbana*-enriched *Artemia* (0.9%) may indicate the occurrence of FA chain elongation (Kanazawa *et al.*, 1979) in phyllosomata, or may point to preferential sequestration. The use of starved *Artemia*, and consequently lower PUFA, may provide a better idea of these abilities in phyllosomata, although possibly at the expense of carbohydrate (Smith *et al.*, 2002a), an important energy source in juvenile *Artemia* (D'Agostino, 1980). The FA profiles in newly-hatched phyllosomata (13% AA, 16% EPA, 10% DHA) are similar to those previously recorded for *J. edwardsii* (12% AA, 15% EPA, 8% DHA) (Phleger *et al.*, 2001).

The MDS scatterplot further shows that early stages of phyllosomata were tightly clustered, while stages IV and V were not. These results highlight two features. First, that there is a change after stage III in FA for these cultured animals, as

with wild phyllosomata, albeit for the cultured animals the change is not seen in the essential long-chain PUFA. Second, the MDS separation in the enriched *Artemia*, compared to the clustering of phyllosomata, and consistency of PUFA profiles (previously outlined) indicates that selected FA in the diets, rather than the PUFA profiles, are transferred through to the larvae. In contrast, the potential prey items of *J. edwardsii* contain a much higher level of DHA (25% mean, 8–39% of total FA) (Nichols *et al.*, 2001); we propose therefore that diets appropriate for cultured phyllosomata have high in DHA.

Conclusions

The examination of the lipid class and FA composition of cultured *J. edwardsii* phyllosomata fed *Artemia* enriched with marine-derived TAG and microalgae has provided insight by which to formulate future enrichment diets designed for rock lobster phyllosomata. This research demonstrates the ability to enrich *Artemia* with DHA via TAG-rich nutrient sources. Feeding the enriched *Artemia* to phyllosomata yielded no TAG and comparatively low DHA. Due to low survival in this feeding trial, we cannot recommend lipid and FA dosages. However, based upon these results and our knowledge that wild phyllosomata (Phleger *et al.*, 2001) and potential prey (Nichols *et al.*, 2001) have low TAG and high DHA, we propose that an appropriate diet for early stage phyllosomata should contain PL oil with a high percentage of DHA. Potential sources of oil having these characteristics include squid and mussel (Nichols *et al.*, 1998c). Such an approach could increase delivery of the key essential PUFA to rock lobster phyllosoma larvae.

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Chapter Four

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FUNCTION OF MOUTHPARTS IN FEEDING BEHAVIOR OF PHYLLOSOMA LARVAE OF THE PACKHORSE LOBSTER, *JASUS VERREAUXI* (DECAPODA; PALINURIDAE)

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ABSTRACT

Stage IV phyllosoma larvae of the packhorse lobster, *Jasus verreauxi* (H. Milne Edwards, 1851), were observed while feeding to determine the processing ability and involvement of mouthparts. Phyllosomata were presented live brine shrimp, *Artemia salina* (Linnaeus, 1758), mussel flesh, *Perna canaliculus* (Gmelin, 1791), and jellyfish, *Aurelia* sp.; and were given chemical or tactile stimulation to induce a feeding response. Larvae were observed tearing their food with maxillipeds 2 and 3, before maceration by maxillipeds 2 and maxillae 1, mastication by mandibles and labrum, and subsequent ingestion of finer particles via a suction action induced by foregut contractions. Phyllosomata became entangled and did not feed when provided soft tissue, such as jellyfish and mussel gonad. Maxilliped action increased when larvae were provided with harder prey, which indicated greater energetic investment required to process the food. Our observations suggest that phyllosomata are capable of processing fleshier prey items than *Artemia,* which is traditionally used as food in artificial culture. An ideal food source for phyllosomata should not readily break up and foul the water, should be free of microbes, and should be energetically economical to capture and masticate.

There is international interest in the culture of rock lobster, however commercial larval cultures have been greatly hindered by a lack of seed lobsters (Jeffs & Hooker, 2000), difficulty in feeding, and limited knowledge of natural food or prey items (Shojima, 1963; Thomas, 1963; Phleger *et al.*, 2001). In order to successfully culture from egg to marketable size, significantly more research is required on the

planktonic larval phase, as this part of the life cycle remains the final obstacle. Considerable effort has and continues to be spent on appropriate aquaria design (Greve, 1968; Illingworth *et al.*, 1997; Kittaka, 1997a; Kittaka & Booth, 2000; Ritar, 2001) and nutritional formulation of aquaculture feeds (Phleger *et al.*, 2001) for phyllosomata, however presentation of feeds and an appropriate delivery system has been neglected.

The lack of suitable diets for phyllosomata is one of the major constraints to the successful culture of palinurid lobsters. Phyllosoma culture has relied on the use of mussel flesh, fish larvae, and enriched *Artemia* as food sources (Kittaka, 1997a). However, some researchers regard *Artemia* as an inappropriate food source due to the labor intensive culture techniques and expense. Furthermore, *Artemia* are a major source of microbial introduction (Blanch *et al.*, 1991; Verdonck *et al.*, 1994; Watanabe *et al.*, 1998), which adversely affects phyllosoma survival (Igarashi *et al.*, 1990; Diggles *et al.*, 2000). To date, inferences about phyllosoma feeding capabilities have been based on examination of the morphology of exanimate and/or preserved lobster larvae (Nishida *et al.*, 1990; Mikami & Takashima, 1994; Rodriguez-Souza *et al.*, 1999; Johnston & Ritar, 2001), however behavioral conclusions must be made with caution when based solely on morphological observations. Although there have been a few general observations of live phyllosomata interaction with prey (Batham, 1967; Kittaka, 1994a; MacMillan *et al.*, 1997), a thorough description of phyllosoma feeding behavior is currently lacking.

Our study builds upon an examination of prey capture and manipulation (Cox, in prep.) and represents the first documentation of the processing ability or food handling and ingestion in live phyllosomata. This may contribute to the clarification of their capabilities to feed both on natural diets and on feed presented in culture situations and is a valuable preliminary step for developing a more appropriate selection of diets and diet characteristics for use in larval culture. Therefore, the aim of our study is to describe mouthpart function, with the objective of determining the roles of each of the limbs and their processing ability.

MATERIALS AND METHODS

Jasus verreauxi phyllosomata were cultured in an upwelling system, as described by Illingworth *et al.* (1997), at 21°C and fed on live cultured *Artemia* until

reaching Stage IV. The ability of phyllosoma mouthparts to deal with a variety of potential items was assessed by handling of the following: live brine shrimp (*A. salina*), fresh mussel (*P. canaliculus*) gonad and adductor muscle, and jellyfish (*Aurelia* sp.). Larvae were also induced to exhibit feeding behavior without prey items, through tactile and chemical stimulation. Tactile stimulation was provided by gently stroking the mechanosensory setae on the pereiopods and mouthpart setation with a fine probe. Chemical stimulation was provided by a seawater solution of dehydrated mussel, *P. canaliculus*, pipetted over the animals. Phyllosomata (n = 30) were dorsally tethered with cyanoacrylic adhesive to fine wire (0.25 mm diameter), as adapted from Alcaraz *et al.* (1980), at the posterior region of the thorax and placed in a glass container of 10–15 ml seawater. Temperature was maintained (21 ± 1°C) by surrounding the chamber with cooled water and light levels were approximately 2 lux. Prey were introduced to phyllosomata with either fine forceps or tethered to a wire and attached to a Narishige (Japan) micromanipulator. Movements of the mouthparts were recorded ventrally, laterally, anteriorly, and dorsally through the transparent exoskeleton. Animals were observed utilizing two video recording-microscopes; a Wild M3C (Heerbrugg, Switzerland) dark field dissecting microscope (10 × 6.4/10/16/40) with a Panasonic WV-CL502 CCD camera, and a Zeiss Standard 20 (West Germany) compound microscope (10 × 2.5/10/40/100) with a JVC Professional CCD camera. The cameras were linked to a JVC TM-150PSN monitor and recorded on a JVC HR-S5000EH video recorder.

RESULTS

Feeding and Mouthpart Function

When prey were provided, the phyllosomata initiated a strong and directed feeding response which allowed pereiopods 1 and 2 (occasionally 3) to adhere to prey using the rigid spinose setae on the distal 4th and 5th segments of the pereiopods, as well as maxillipeds 2 and 3. Prey items were manipulated and pushed towards the oral region (Fig. 1), where the endopodites of maxillipeds 3 exhibited an articulated hooking action for additional control over prey. Maxillipeds 2 displayed a deliberate shredding/tearing action and pushed torn pieces of food towards maxillae1 (Fig. 1). Since maxillipeds 2 and 3 exhibited a wide range of motion, they effectively in-

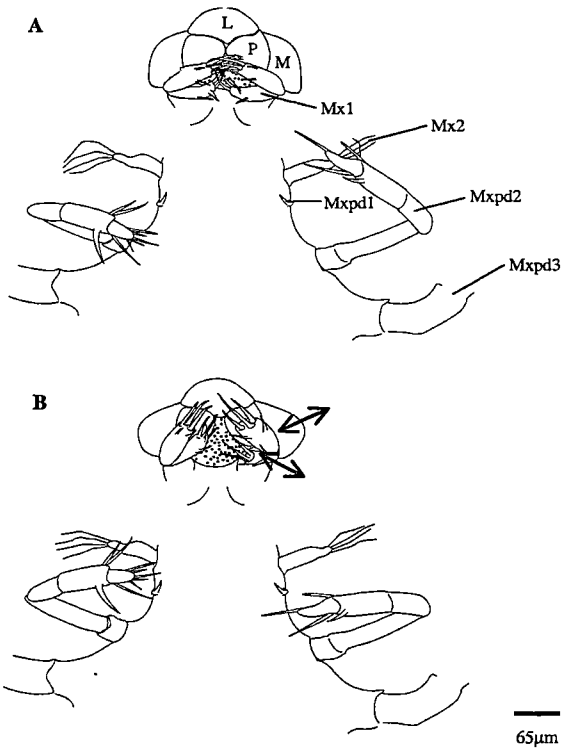

Fig. 1. Mouthparts of stage IV *Jasus verreauxi* phyllosoma. A. Oral region showing closed position of mouthparts. B. Oral region showing opened position of mouthparts. Mandibles separate to allow mastication of food particles by molar process (not shown). Paragnaths remain stationary to hold food particles. Arrows indicate direction of movement by mandibles and maxillae 1. Scale, 65 µm. L, labrum; M, mandible; P, paragnath; Mx1, maxilla 1; Mx2, maxilla 2; Mxpd1, maxilliped 1; Mxpd2, maxilliped 2; Mxpd3, base of maxilliped 3.

creased the oral field. Maxillipeds 1 were not used for feeding and were represented by a rudimentary spine. The small setose maxillae 2 beat continuously during feeding (periodically changing frequency) in a dorso-ventrally oriented direction. This motion did not appear to facilitate the entrapment of smaller particles for feeding purposes. The maxillae 1 have independently operating projections, which push food towards the mouth (and between the mandibles) at a 45° angle (relative to the transverse plane) and retract to the margins of the paragnath (Fig. 1). The endites open at the mouth to release food particles, which are drawn into the foregut chamber via a suction action induced by foregut contractions (Fig. 1). The maxillae 1 also rubbed across the surface of the paragnaths where mucus secreted from tegumental glands was taken into the mouth to aid in ingestion (Mikami & Takashima, 1993, 1994).

The paragnaths exhibited very limited movement, which was independent of the mandibles, however, they did aid in gripping and trapping food particles placed into the mouth by the maxillae 1. Mandibles operated on a 45° angle relative to the

transverse plane, shearing against the posterior region of the labrum (Fig. 1). Sharp incisor and molar processes on the mandibles cut and tore food and aided the maxillae 1 in that external mastication role. Scanning electron microscopy images detailing the incisor and molar processes are available for *Jasus verreauxi* (D. J. Johnston, pers. comm.) and *J. edwardsii* (Hutton, 1875) (Nishida *et al.*, 1990; Johnston & Ritar, 2001).

Throughout the feeding process, foregut and midgut peristaltic contractions were evident and created an inward suction allowing food to be ingested. Small particles were observed passing from the diverticulum to the hindgut, where they formed a series of elongate waste boluses (the length was approximately 1.5–2 times the width) attached by a mucal thread. Particles took approximately 4–5 minutes to pass from mouth through the gut and be egested.

Processing Prey Items

When fed *Artemia*, the phyllosomata rapaciously shredded them with maxillipeds 2 and 3 and maxillae 1 prior to ingestion of small particles. Phyllosomata easily tore apart mussel adductor muscle tissue and ingested material. Less activity was observed for maxillipeds 2 and 3 and maxillae 1 when feeding upon mussel than when feeding upon *Artemia*. When fed mussel gonad, the phyllosomata became quickly fouled on all appendages. Rather than actively ingesting the food, phyllosomata attempted cleaning for an extended period to remove the material. With jellyfish, as with the mussel gonad, the phyllosomata quickly shredded it and became entangled in the material.

No Feeding Stimulation

Phyllosomata without stimulation exhibited limited activity of pereiopod endopods, however the setose exopods beat continuously. The endopods of maxillipeds 3 continuously cleaned the spinose projections at the base of the pereiopods and the surfaces of maxillipeds 1 and 2. Maxillipeds 2 and the endopods of maxillipeds 3 also exhibited cleaning action, although there was no apparent debris on the setae or exoskeleton. The maxillae 2 remained beating with a fairly constant frequency and did not appear to exhibit any other function that may be associated with feeding. The mandible, paragnaths, and labrum remained unresponsive, however there was slight foregut contraction and very slight gut movement.

Tactile Stimulation

Contact with the pereiopods by the probe elicited an immediate grab response by all pereiopods directed toward the probe. This action was followed by anteriorly directed pereiopod movement. Subsequently, maxillipeds 3 attempted to grasp the probe and pass it forward to the smaller mouthparts. Maxillipeds 2 exhibited a seemingly non-directed, anticipatory movement. The endites of the maxillae 1 exhibited a slight opening action. Mandibles and paragnaths were unresponsive unless physically touched by the probe, which resulted in a brief frenzy of lateral movements similar to feeding activity.

Chemical Stimulation

All pereiopods exhibited an immediate grab response directed toward the oral region. Maxillipeds 3 displayed a searching/anticipating action within the oral region. That is, while the pereiopods made a direct grab in front of the mouth, maxillipeds 3 moved around the oral region. The feeding response induced by chemical stimulation was generally more instantaneous compared with tactile stimulation. Considerably more mandible, paragnath and maxillae 1 action was induced by the addition of a chemical stimulus. Foregut contractions appeared more frequent and intense compared with contractions exhibited during tactile stimulations.

DISCUSSION

Since the mouthparts of *J. verreauxi* are similar to other spiny lobster species, our results have wide applicability, and can be compared to research based on mouthpart descriptions (e.g., Johnston & Ritar, 2001). Although previous suppositions suggest that phyllosomata are limited to soft tissues by mouthpart and foregut morphology (Johnston & Ritar, 2001) and lack of gastric mill (MacMillan *et al.*, 1997), we observed the prey to be processed exteriorly. It does not appear that the mandibles limit phyllosoma to prey of a particular hardness, as the maxillipeds effectively shred a range of food items of different hardness into smaller particles prior to entry into the mouth cavity. Even the relatively hard prosome of copepods were penetrated (Cox, pers. comm.). Therefore, phyllosomata may only be limited by their ability to capture and manipulate prey.

The effort of shredding the moderately hard *Artemia* carapace with maxillipeds 2 and 3 and maxillae 1 may be energetically costly. This represents an energy expen-

diture in addition to capturing prey. Alginate (pizza) diets contain *Artemia* (Tong *et al.*, 1997), so although that diet may be less energetically demanding on phyllosoma by minimizing 'prey capture' time, phyllosomata must still expend excessive energy to shred the carapace.

In the present study, the mussel adductor muscle proved to be a manageable texture for phyllosomata, while the use of mussel gonad caused fouling of phyllosoma appendages. Success in on-growing phyllosomata until pueruli has been limited, and greatest rates of survival and metamorphosis were achieved when feeding mussel, *Mytilus edulis* (Linnaeus, 1758) (Kittaka, 1994a, 1997b). However, these studies used mussel gonad, not adductor mussel, and the addition of mussel to aquaria reduced water quality by increasing ammonia-N and bacterial number (Kittaka, 1994a). Although the addition of the microalga *Nannochloropsis* sp. into culture has been shown effectively to control both (Kittaka, 1994a), microalgal culture is labor intensive and may not be economically feasible.

In contrast to the reported association of jellyfish with *Panulirus interruptus* (Randall) phyllosomata (Mitchell, 1971), scyllarid species (Herrnkind *et al.*, 1976) and cultured phyllosomata (Kittaka, 1994b), and conclusions that mouthparts are adapted for gelatinous zooplankton (MacMillan *et al.*, 1997; Johnston & Ritar, 2001), the evidence presented here for *Jasus verreauxi* here indicates that jellyfish may be an unsuitable food source. Granted, our trials with a single lobster species cannot completely represent all Palinura. However, since jellyfish fouled the phyllosomata during these trials, we propose a more stable feed would be appropriate. Additionally, gelatinous zooplankton are of low nutritional value (Kittaka, 1994b), consisting of mostly water and low percent total lipid (Nelson *et al.*, 2000). They may suffice during opportunistic feeding of wild phyllosomata, but, we suggest, would be inadequate for aquaculture. Lastly, use of live feeds, as with *Artemia* (Blanch *et al.*, 1991; Verdonck *et al.*, 1994; Watanabe *et al.*, 1998), introduces a bacterial load.

In conclusion, we suggest that neither jellyfish nor mussel gonad are suitable feeds for phyllosomata in aquaculture since they unduly foul larval appendages. Additionally, use of *Artemia* and mussel flesh is not optimal since the aim in culture water is to reduce bacterial load. We can, however, aim to formulate a feed with the more positive qualities of mussel, such as soft but stable texture and high nutritional levels. Since phyllosomata respond well to both tactile and chemical stimulation, it

may not be necessary to provide both cues in a single formulation. As it may be difficult to formulate a feed that both retains nutrients and releases chemical stimuli, we propose that a formulated feed may not require chemical attractants. An appropriate formulated feed should be free of microbes, firm, fleshy, and in discrete pieces that are not sticky or adherent; a diet that can be readily caught, manipulated, and ingested by rock lobster phyllosomata.

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Chapter Five

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FEEDING SOUTHERN ROCK LOBSTER, *JASUS EDWARDSII* HUTTON, 1875, PHYLLOSOMATA IN CULTURE: RECENT PROGRESS WITH LIPID-ENRICHED *ARTEMIA*

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ABSTRACT *Jasus edwardsii* phyllosoma larvae were successfully on-grown in static culture with antibiotics from newly-hatched to stage V with high survival. Feeding phyllosomata on *Artemia salina* Linnaeus, 1758, enriched with (1) a triacylglycerol (TAG)-rich A1 DHA Selco–*Chaetoceros muelleri* Lemmermann, 1898, nutrient source or (2) a formulated ethyl ester (EE)-rich nutrient source was compared to the more novel approach of using a formulated mussel powder–polar lipid diet attached to mesh. Individuals showed an increase to stage V in dry mass (0.1 to 1.5 mg) and total length (2.1 to 6.1 mm). Survival of *Artemia*-fed phyllosomata was high (92–98% from stages II–III; 49% mean total survival). Animals fed the mussel powder–polar lipid diet had low molt success, although the presence of faecal trails confirmed they were consuming the diet. Total lipid remained generally constant in *Artemia*-fed phyllosomata from newly-hatched to stage V (155 mg g^{-1} dry mass); this was notably higher than observed for previous feeding trials. The major lipid class in all phyllosomata samples was polar lipid, followed by sterol, with TAG as a minor component only, and EE not detected. The main fatty acids (FA) were 18:1(n-9)c, 18:2(n-6), 16:0, eicosapentaenoic acid [EPA; 20:5(n-3)], 18:0, 18:1(n-7)c, arachidonic acid [AA; 20:4(n-6)] and docosahexaenoic acid [DHA; 22:6(n-3)]. Levels of the essential polyunsaturated fatty acids (PUFA), namely, AA, EPA and, in particular, DHA, decreased, on both a relative and absolute basis, from newly-hatched to stage V, although phyllosomata fed the EE-rich enriched *Artemia* diet showed higher essential PUFA content together with oil content. This experiment further validates that lipids and fatty acids are important nutritional component in rock lobster larvae and that feeding phyllosomata with lipid-enriched *Artemia* maintains excellent growth and survival in early stages. Strategies will be needed, however, to either

overcome the issue of low DHA, in particular, delivered by *Artemia* (due to retro-conversion), or to supply DHA by alternate means at later stages.

KEY WORDS: *Artemia*; enrichment; fatty acids; *Jasus edwardsii*; lipids; lobster; phyllosoma

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INTRODUCTION

Rock lobster in Australasia has recently attracted the interest of number of research institutions for its potential as a valuable aquaculture species. The fishery for southern rock lobster, *Jasus edwardsii* Hutton, 1875, boasts a value of over A$200 million in Australia (Punt & Kennedy, 1997) and NZ$100 million in New Zealand (Breen & Kendrick, 1997). As wild fishing pressure escalates (Booth & Phillips, 1994), future exploitation of the rock lobster marketplace will logically be realized through aquaculture (Phleger *et al.*, 2001).

As an aquaculture species, rock lobster possesses the allure of potentially high financial reward. Equally great is the challenge for research scientists, since the larval phase, including metamorphosis from phyllosoma to puerulus, is extensive (Phillips & Sastry, 1980; McWilliam & Phillips, 1997), currently requiring close to a year in culture (Tong *et al.*, 2000a). To conquer this challenge, several vital aspects of culture of rock lobster phyllosomata can be identified as follows: (1) exploration of feeding capabilities of phyllosomata (Johnston & Ritar, 2001; Nelson *et al.*, 2002a) to determine appropriate format of feed presentation; (2) determination of nutritional requirements to focus further the feed format; (3) suitable aquarium design (Kittaka & Booth, 2000; Ritar, 2001) to optimize exposure of animals to the food source while minimizing microbial loading (Igarashi *et al.*, 1990; Diggles *et al.*, 2000).

This study examines the second aspect (noted above), nutrition, and in particular the requirements for lipids. To focus this aspect, features of lipid nutrition under examination include: (1) total lipid content, the mg g^{-1} of the lipid provided in the diet and that incorporated into larvae; (2) the lipid classes, examination of the delivery and incorporation of types of lipids such as TAG, polar lipid (PL) and EE; (3) the profile of FA, which are components of lipid classes.

Building on the studies of lipids and FA in wild phyllosomata (Phleger *et al.*, 2001) and potential prey items (Nichols *et al.*, 2001), we have examined enrichment of *Artemia* with essential PUFA (Phleger *et al.*, 2001; Nelson *et al.*, 2002b; Smith *et al.*, 2002a) and feeding of these TAG-enriched *Artemia* to phyllosomata (Nelson *et al.*, 2003b). The evidence amassed to date from these studies indicates that wild phyllosomata largely obtain, and therefore may require lipid in a PL form, rather than in a TAG form. However, *Artemia* store their lipid enrichment as TAG (McEvoy *et al.*, 1996; Sorgeloos *et al.*, 1998; Harel *et al.*, 1999). With this in mind, and since phyllosomata do consume static food items (e.g., mussel pieces) (Kittaka, 1997b; Matsuda & Yamakawa, 2000; Nelson *et al.*, 2002a), the present study was performed to provide phyllosomata a diet presented on a feed station (i.e., formulated diet attached to aquaria), a format currently receiving attention (Cox & Johnston, 2003c). A comparison was made for feed-station fed larvae to animals fed *Artemia*, enriched with either a TAG-rich product or with a novel DHA-rich EE product, by examining the effects on *J. edwardsii* phyllosomata survival, growth and lipid composition.

METHODS

Artemia Enrichment

Decapsulated *Artemia* cysts (INVE, Great Salt Lake Prime Gold) were hatched at 28 ± 1°C in 50-L white fiberglass cones in 0.2-μm filtered brackish water (27 ± 1 g kg^{-1}), with vigorous aeration and a 150 W light suspended 0.5-m above the water. After 24 h, *Artemia* nauplii were removed from the hatching cones, rinsed in freshwater for 2 min and transferred into 1000-L tanks of filtered seawater (0.2 μm, 34 ± 1 g kg^{-1}, 27 ± 1°C). *Artemia* were fed twice daily with a rice pollard–soy flour–wheat flour brine shrimp diet (Eyre Peninsula Aquafeeds, South Australia, Australia) at a rate to maintain a Secchi depth of 25–30 cm. The environmental parameters remained stable for the duration of the on-growing period; salinity (35.7 ± 0.2 g kg^{-1}), pH (8.3 ± 0.0), dissolved oxygen (7–7.2 mg l^{-1}) and temperature (26.9 ± 0.1°C). After 5 days, 80,000 *Artemia*, with a total length of 1.5 ± 0.2 mm, were removed from the on-growing container, rinsed in freshwater for 2 min and transferred to the 50-L white fiberglass cones containing 10-L of filtered seawater to achieve a density of 4 ml^{-1}.

Artemia were enriched for 24 hrs with 0.6 g L^{-1} of three nutrient sources (i.e., *Artemia* enrichment diets):

(1) A1 DHA Selco (INVE Group, Belgium),

(2) the microalga *Chaetoceros muelleri* Lemmermann, 1898,

(3) Ethyl ester–mussel: a mixture of New Zealand Greenshell mussel [*Perna canaliculus* Gmelin, 1791] powder (NIWA Research, Auckland, New Zealand)–DHA (66%) EE oil (CSIRO Marine Research, Hobart, Australia)–AA (39%) TAG marine oil (Sun-TGA40S, Suntory Limited, Osaka, Japan)–Greenshell mussel polar lipid (NIWA Research, Auckland, New Zealand) (56:30:10:4 by mass).

C. muelleri were cultured and the cell density was measured daily as described in Wilkinson (2000). The non-algal enrichment diets were prepared daily by homogenizing ingredients suspended in seawater.

Experimental Aquaria

Three hundred phyllosomata $aquarium^{-1}$ were on-grown from newly-hatched to stage V in 3-L plastic static aquaria on three diet treatments; each treatment was conducted in triplicate. The diet treatments consisted of:

(1) *Artemia* enriched with A1 DHA Selco and *Artemia* enriched with *C. muelleri* (1:2 v/v),

(2) *Artemia* enriched with the Ethyl ester–mussel nutrient source (as described above),

(3) Mussel powder–polar lipid feed station diet [Greenshell mussel powder–Greenshell mussel polar lipid–lyprinol (from Greenshell mussel) (NIWA Research, Auckland, New Zealand)–sodium alginate (81:10:5:4 by mass)] affixed to 8 × 17 cm meshes (bird netting) with 10% $CaCl_2$ solution.

The water in aquaria was changed daily. After recording any molts/mortalities, the contents of each aquarium was poured through a 1000-μm screen, retaining the phyllosomata whilst the uneaten feed and debris went to waste. The aquaria were cleaned, refilled with seawater and larvae were washed back in. Phyllosomata were provided with new diets once daily in the afternoon. *Artemia* were fed to phyllosomata at a rate of 3 *Artemia* ml^{-1}. Oxytetracycline was added to the water at 20 mg L^{-1} daily. After each molt, all animals were counted and 10 phyllosomata $aquarium^{-1}$

were measured for total length, carapace length and carapace width utilizing a dissecting microscope, digital camera and Scion Image Beta 4.0.2 software (Scion Corporation, Frederick, Maryland, USA).

Lipid Extraction

Artemia and phyllosomata samples were filtered through 4.7-cm Whatman glass filters (GF/F) and rinsed with 0.5 M ammonium formate. Sample numbers of phyllosoma taken for lipid analyses were: 400 newly-hatched (sampled at start prior to distribution of larvae to aquaria); from each aquarium 50 stage II, 35 stage III, 25 stage IV and 15 stage V; all midstage. Samples were lyophilized to determine dry mass and quantitatively extracted overnight using a modified Bligh and Dyer (1959) one-phase methanol–chloroform–water extraction (2:1:0.8 v/v/v). The phases were separated by the addition of chloroform–water (final solvent ratio, 1:1:0.9 v/v/v methanol–chloroform–water). The total solvent extract (TSE) was concentrated using rotary evaporation at 40°C.

Lipid Classes

An aliquot of the TSE was analyzed using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to quantify individual lipid classes (Volkman & Nichols, 1991). Samples were applied in duplicate to silica gel SIII chromarods (5-μm particle size) using 1-μl micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The primary solvent system used for the lipid separation was hexane–diethyl ether–acetic acid (60:17:0.1), a mobile phase resolving non-polar compounds such as wax ester (WE), TAG, free fatty acids (FFA) and sterols (ST). A second non-polar solvent system of hexane–diethyl ether (96:4) was also used to resolve hydrocarbons, WE, TAG and diacylglyceryl ether (DAGE). After development, the chromarods were oven dried and analyzed immediately to minimize absorption of atmospheric contaminants. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl oleate, oleic acid, squalene, TAG (derived from fish oil), WE (derived from orange roughy oil) and DAGE (derived from shark liver oil); 0.1–10 μg range]. Peaks were quantified on an IBM compatible computer using DAPA Scientific software (Kalamunda, Western Australia, Australia). TLC-FID results are

generally reproducible to ±5–10% of individual class abundances (Volkman & Nichols, 1991).

Fatty Acids

An aliquot of the total lipid was *trans*-methylated to produce fatty acid methyl esters (FAME) using methanol–chloroform–conc. hydrochloric acid (10:1:1, 80°C, 2 hr). FAME were extracted into hexane–chloroform (4:1, 3 × 1.5 ml) and treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA 50 μl, 70°C, overnight) to convert ST and alcohols to their corresponding TMSi ethers.

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC (Avondale, PA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Helium was the carrier gas. Following addition of methyl nonodecanoate and methyl tricosanoate internal injection standards, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1}, and finally to 300°C at 5°C min^{-1}. Peaks were quantified with Waters Millennium software (Milford, Massachusetts, USA). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, Texas, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

RESULTS

Morphometrics

The increase in total length and mass in phyllosomata was similar between the two *Artemia* diet treatments, with a good fit of exponential trend lines observed ($R^2 \geq$ 0.999; Fig. 1). Phyllosomata fed the A1 DHA Selco–*C. muelleri-Artemia* diet treatment showed a greater increase in total length (2.1 to 6.1 mm) and mass per individual (0.1 to 1.5 mg dry mass) from stages I to V, than did larvae fed Ethyl ester–mussel-enriched *Artemia* (total length: 5.9 mm; mass per individual: 1.2 mg dry

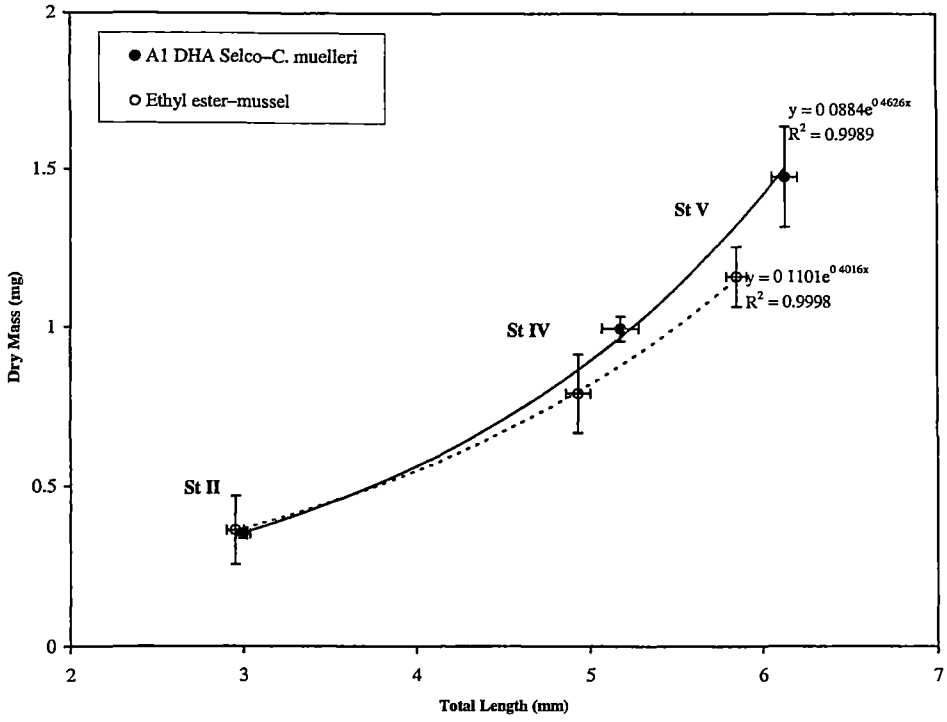

Figure. 1. Dry mass as a function of total length of *Jasus edwardsii* phyllosomata from stages I to V on two diet treatments of *Artemia* enriched with either A1 DHA Selco–*C. muelleri* or Ethyl ester–mussel nutrient sources. Presented as mean ± SD; fitted with exponential trend lines.

mass; Fig. 1). Percentage survival was >68% between each stage, and was highest from stages II–III (92–98%; Table 1). Total survival to stage V was high for animals fed *Artemia* enriched with either the A1 DHA Selco–*C. muelleri* (57%) or Ethyl ester–mussel (42%) nutrient sources. There were no differences in intermolt period for *Artemia*-fed phyllosomata among treatments. Intermolt periods were 9, 11, 12 and 13–15 days to commencement of molt for stages I–II, II–III, III–IV, IV–V, respectively.

Phyllosomata fed the Mussel powder–polar lipid diet failed to molt to stage II on the feed station diet alone. Two animals remained alive at stage I for 30 days, at which time they were put on the Ethyl ester–mussel-enriched *Artemia* diet. At day 41 they successfully molted to stage II, and were sampled at day 56. After sampling stage II animals at day 15, phyllosomata fed A1 DHA Selco–*C. muelleri*-enriched *Artemia* were divided and half were put on the Mussel powder–polar lipid diet. After 10 days, these animals molted to stage III, were sampled at day 30, but failed to molt to stage IV. After sampling stage IV animals at day 37, phyllosomata fed Ethyl ester–mussel-enriched *Artemia* were divided and half were put on the Mussel powder–

TABLE 1.
Intermolt period (days) and percentage survival of phyllosomata fed different diets.

	Diet		
	A1 Selco–*C. muelleri*[b]	Ethyl ester–mussel[b]	Mussel powder–polar lipid[c]
Survival[a]			
I–II	81.8 ± 4.8	76.2 ± 15.9	–
II–III	97.5 ± 2.2	92.3 ± 3.5	93.5 ± 7.2
III–IV	87.3 ± 6.0	86.0 ± 6.7	51.3 ± 3.6
IV–V	81.1 ± 14.5	68.3 ± 6.8	–
Total	56.5 ± 12.6	41.5 ± 11.2	–
Intermolt period			
I–II	9	9	–
II–III	11	11	10
III–IV	12	12	–
IV–V	13	15	–

[a] Presented as mean ± SD; $n = 3$; [b] Enriched *Artemia*; [c] Feed station.

polar lipid diet. They did not molt to stage V, but were sampled concurrently with *Artemia*-fed phyllosomata at day 56.

Lipid Content and Classes

The two nutrient sources were lipid-rich, with A1 DHA Selco higher than Ethyl ester–mussel (960 and 410 mg g^{-1} dry mass, respectively; Table 2). A1 DHA Selco was dominated by TAG (88%) and Ethyl ester–mussel by EE (55%), with TAG the second most abundant lipid class (28%). Lipid content of *Artemia* enriched with A1 DHA Selco–*C. muelleri* and Ethyl ester–mussel was identical (250 mg g^{-1} dry mass). TAG was the major lipid class (46–51% of total lipid), followed by PL (37–40%). ST (5–6%), FFA (4–10%), diacylglycerol (DG; 0.7–1.9%) and WE (0.1–0.3%) were minor components.

In *Artemia*-fed phyllosomata, although lipid per individual generally increased from newly-hatched to stage V (8 to 180 μg), the absolute lipid content remained generally constant in *Artemia*-fed phyllosomata from newly-hatched to stage V (Table 2). Total lipid was 155 mg g^{-1} dry mass in newly-hatched phyllosoma and increased slightly from stage I to stage II (207 and 173 mg g^{-1} for A1 DHA Selco–*C. muelleri* and Ethyl ester–mussel *Artemia*-fed phyllosomata, respectively) and to stage IV (157 and 176 mg g^{-1}). By stage V, total lipid decreased to the starting (newly-hatched) value in Ethyl ester–mussel *Artemia*-fed phyllosomata (156 mg g^{-1}) and was slightly lower in animals fed A1 DHA Selco–*C. muelleri*-enriched *Artemia*

TABLE 2.
Percentage lipid class composition of nutrient sources, enriched *Artemia*, feed station and phyllosomata.

	Wax ester	Ethyl ester	Triacylglycerol	Free fatty acid	Diacylglycerol	Sterol	Polar lipid	Lipid as mg g^{-1} dry mass	Lipid mass $indiv^{-1}$ dry mass (μg)
Nutrient souces									
A1 DHA Selco	0.0 ± 0.0	–	88.2 ± 0.8	1.6 ± 0.1	–	0.8 ± 0.0	9.5 ± 0.9	958.5 ± 18.7	–
Ethyl ester–mussel	0.7 ± 0.0	55.1 ± 5.8	27.9 ± 0.7	5.4 ± 1.6	0.1 ± 0.0	1.4 ± 0.4	9.3 ± 3.1	411.9 ± 44.2	–
Artemia									
A1 DHA Selco–*C. muelleri*	0.1 ± 0.0	–	45.9 ± 0.4	10.0 ± 0.2	1.9 ± 0.3	5.6 ± 0.0	36.5 ± 1.0	259.7 ± 12.8	–
Ethyl ester–mussel	0.3 ± 0.2	–	50.5 ± 0.2	3.5 ± 0.5	0.7 ± 0.1	5.2 ± 0.0	39.7 ± 0.9	254.2 ± 0.3	–
Feed station									
Mussel powder–polar lipid	2.8 ± 0.1	–	12.5 ± 0.3	26.2 ± 0.1	0.4 ± 0.1	3.5 ± 0.3	54.6 ± 0.9	191.0 ± 11.4	–
Phyllosomata									
Newly-hatched	1.7 ± 0.1	–	0.2 ± 0.1	9.9 ± 0.1	–	9.9 ± 0.1	78.3 ± 0.1	154.6 ± 9.4	8.0 ± 1.1
A1 DHA Selco–*C. muelleri*[a]									
II	–	–	–	5.5 ± 5.0	1.5 ± 0.4	4.3 ± 1.7	88.7 ± 6.8	207.5 ± 19.6	72.7 ± 6.1
IV	0.3 ± 0.0	–	0.2 ± 0.1	4.7 ± 0.6	3.9 ± 0.7	6.9 ± 0.2	84.1 ± 1.4	157.2 ± 13.9	156.0 ± 8.0
V	0.3 ± 0.1	–	0.3 ± 0.1	2.1 ± 0.2	3.0 ± 0.3	7.1 ± 0.6	87.2 ± 0.9	127.8 ± 9.5	188.9 ± 25.2
Ethyl ester–mussel[a]									
II	–	–	–	8.1 ± 3.4	1.5 ± 0.0	5.5 ± 0.4	84.8 ± 3.7	173.0 ± 17.5	61.3 ± 13.3
IV	0.5 ± 0.3	–	0.2 ± 0.1	4.9 ± 0.8	3.0 ± 0.4	6.3 ± 0.4	85.1 ± 1.1	175.6 ± 18.4	137.3 ± 8.3
V	0.5 ± 0.1	–	0.4 ± 0.1	2.3 ± 0.5	3.2 ± 0.2	7.5 ± 0.4	86.1 ± 1.3	156.0 ± 19.3	180.0 ± 13.3
Mussel powder–polar lipid[b]									
III	2.7 ± 0.2	–	1.1 ± 0.1	9.2 ± 1.2	0.8 ± 0.3	12.8 ± 0.8	73.4 ± 0.9	54.2 ± 7.5	46.7 ± 10.8
IV	1.0 ± 0.3	–	0.5 ± 0.3	2.7 ± 0.3	2.2 ± 0.9	10.1 ± 1.9	83.4 ± 2.5	155.5 ± 86.4	133.3 ± 57.0

Presented as mean ± SD; $n = 3$; (–), below detection; [a] Enriched *Artemia*; [b] Feed station (molted from stage II to III only).

(128 mg g^{-1}). PL comprised the major lipid class in all phyllosoma samples (73–87% of total lipid), followed by ST (4–8%; mainly cholesterol), FFA (2–8%), DG (2–4%) and WE (0–0.5%). Minor TAG was detected (0–0.4%).

Stage IV phyllosomata fed the Mussel powder–polar lipid diet were similar to those fed either *Artemia* diet, with 156 mg g^{-1} dry mass of lipid and PL the dominant lipid class (83%; Table 2). ST was comparatively higher (10%). Compared to other phyllosoma, PL (73%) and DG (1%) were lower in stage III Mussel powder–polar lipid feed station-fed animals, with a proportionate increase in ST (13%) and WE (3%). Lipid content was a third that of other *Artemia*-fed samples (54 mg g^{-1} dry mass).

Fatty Acids

The FA in the two nutrient sources differed markedly (Table 3). In A1 DHA Selco, dominant FA in decreasing order of proportional abundance of total FA were: palmitic acid (16:0; 17%), EPA (15%), oleic acid [18:1(n-9)c; 14%], palmitoleic [16:1(n-7)c, 9%], DHA (8%), myristic acid (14:0; 7%) and linoleic acid [18:2(n-6); 5%]. The Ethyl ester–mussel nutrient source was dominated by PUFA (75%), with major FA as: DHA (37%), AA (13%), EPA (12%) and 16:0 (7%).

The major FA in enriched *Artemia* were: 18:1(n-9)c (32–36%), 18:2(n-6) (23–27%), 16:0 (9–11%), *cis*-vaccenic acid [18:1(n-7)c; 4%], stearic acid (18:0; 4%) and 16:1(n-7)c (2–4%; Table 3). *Artemia* enriched with Ethyl ester–mussel had higher essential PUFA (3% AA, 6% EPA, 7% DHA) than those enriched with A1 DHA Selco–*C. muelleri* (1% AA, 2% EPA, 1% DHA). The Mussel powder–polar lipid diet was dominated by 16:0 (19%), EPA (14%) and DHA (14%), with AA at 3% of total FA. Compared to *Artemia*, levels in the Mussel powder–polar lipid diet of 18:0 fatty aldehyde (6%), 20:1(n-9)c (4%) and minor C_{22} PUFA (3%) were elevated, and levels of 18:1(n-9)c (3%) and 18:2(n-6) (2%) were lower.

In *Artemia*-fed phyllosomata, the major FA were similar to those found in the enriched *Artemia* and in decreasing order of abundance were: 18:1(n-9)c (23–27% of total FA), 18:2(n-6) (17–22%), 16:0 (9–11%), 18:0 (7–9%), EPA (7–11%), 18:1(n-7) (4–6%), DHA (4–6%) and AA (2–5%%; Table 4). These phyllosomata experienced a decrease in essential PUFA, on both a relative (Table 4) and absolute basis (Fig. 2), from newly-hatched to stage V. In phyllosomata fed Ethyl ester–mussel-enriched

TABLE 3.
Percentage fatty acid composition of nutrient sources, enriched *Artemia* and feed station.

	Nutrient sources		*Artemia*		Feed station
	A1 DHA Selco	Ethyl ester–mussel	A1 DHA Selco–*C. muelleri*	Ethyl ester–mussel	Mussel powder–polar lipid
14:0	6.9 ± 0.2	0.7 ± 0.1	1.3 ± 0.1	0.5 ± 0.0	3.7 ± 0.3
16:1(n-7)c	8.8 ± 1.0	0.8 ± 0.1	3.9 ± 0.1	2.0 ± 0.0	5.3 ± 0.4
16:0	16.6 ± 0.0	7.2 ± 0.8	11.0 ± 0.2	9.1 ± 0.1	18.5 ± 1.2
18:4(n-3)	2.7 ± 0.0	1.5 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	2.1 ± 0.1
18:2(n-6)	5.1 ± 0.2	3.6 ± 0.3	27.2 ± 0.3	22.9 ± 0.3	1.9 ± 0.1
18:1(n-9)c/18:3(n-3)	14.1 ± 0.8	3.5 ± 0.7	35.8 ± 0.2	31.8 ± 0.6	2.6 ± 0.1
18:1(n-7)c	3.0 ± 0.1	0.5 ± 0.0	4.1 ± 0.0	3.7 ± 0.1	2.6 ± 0.1
18:0	3.5 ± 0.2	3.4 ± 0.3	4.2 ± 0.0	4.4 ± 0.1	5.1 ± 0.0
18:1 Falde	0.1 ± 0.0	–	–	0.7 ± 0.8	6.4 ± 0.1
20:4(n-6)	0.9 ± 0.0	13.2 ± 0.6	0.5 ± 0.0	3.4 ± 0.0	2.7 ± 0.1
20:5(n-3)	14.9 ± 0.6	12.3 ± 0.3	2 4 ± 0.3	6.3 ± 0.0	13.9 ± 0.4
20:4(n-3)	0.1 ± 0.0	0.1 ± 0.0	0.9 ± 0.0	1.4 ± 0.1	2.1 ± 0.1
20:1(n-9)c	0.4 ± 0.4	0.4 ± 0.5	0.0 ± 0.0	0.8 ± 0.1	3.6 ± 0.2
22:6(n-3)	7.9 ± 0.3	37.1 ± 0.7	0.8 ± 0.1	7.0 ± 0.6	13.5 ± 0.9
C_{22} PUFA	–	0.7 ± 0.0	–	–	2.9 ± 0.3
Other	14.8	15.0	7.6	5.7	13.0
Sum SFA	31.8 ± 0.2	15.9 ± 0.4	18.9 ± 0.3	16.5 ± 0.1	31.6 ± 1.6
Sum MUFA	30.7 ± 1.5	8.5 ± 1.2	47.0 ± 0.6	40.8 ± 0.1	18.1 ± 0.3
Sum PUFA	37.4 ± 1.3	74.7 ± 0.2	33.7 ± 0.6	42.4 ± 0.5	43.1 ± 1.8
Sum (n-3)	28.3 ± 1.0	52.9 ± 0.9	3.7 ± 0.3	14.0 ± 0.7	32.8 ± 1.5
Sum (n-6)	7.0 ± 0.1	20.6 ± 1.0	28.2 ± 0.5	27.5 ± 0.5	6.5 ± 0.1
Ratio (n-3)/(n-6)	4.1	2.6	0.1	0.5	5.0
Ratio EPA/AA	15.8	0.9	5.2	1.9	5.1
Ratio DHA/EPA	0.5	3.0	0.3	1.1	1.0

Presented as mean ± SD; $n = 3$; (–), below detection, AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Other includes components present at <2%: i15:0, a15:0, 15:0, i16:0, C_{16} PUFA, 16:1(n-9)c, 16:1(n-7)t/16:2, 16:1(n-5)c, 16:0 Falde (fatty aldehyde), 16:1 Falde, i17:0, a17:0, 17:1, 17:0, 18:3(n-6), i18:0, 18:1(n-7)t, 18:1(n-5)c, 18:0 Falde, i19:0, 19:1, 20:3(n-6), 20:2(n-6), 20:1(n-11)c, 20:1(n-9)c, 20:1(n-7)c, 20:0, C_{21} PUFA, 21:0, 22:5(n-6), 22:4(n-6), 22:5(n-3), 22:1(n-11), 22:1(n-7), 22:0, 24:1, 24:0.

TABLE 4.
Percentage fatty acid composition of phyllosomata from feeding trial.

		Diet								
		A1 DHA Selco–*C. muelleri*[a]			Ethyl ester–mussel[a]			Mussel powder–polar lipid[b]		
	New hatch	II	IV	V	II	IV	V	II[3]	III	IV
16:1(n-7)c	4.1 ± 0.2	2.7 ± 0.1	1.8 ± 0.0	3.1 ± 0.2	1.6 ± 0.0	0.9 ± 0.0	1.6 ± 0.2	2.1	2.1 ± 0.4	0.9 ± 0.0
16:0	12.2 ± 0.4	10.7 ± 0.5	10.0 ± 0.0	11.3 ± 0.1	10.4 ± 0.0	9.0 ± 0.2	10.1 ± 0.3	15.4	15.1 ± 2.2	12.2 ± 1.0
17:0	1.6 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	0.7 ± 0.1	4.6	1.3 ± 0.2	0.9 ± 0.1
18:2(n-6)	0.7 ± 0.0	17.4 ± 0.4	21.7 ± 0.2	18.3 ± 0.3	17.1 ± 0.1	20.1 ± 0.0	18.3 ± 0.3	9.7	5.4 ± 0.8	13.0 ± 2.4
18:1(n-9)c/18:3(n-3)	8.1 ± 0.2	26.5 ± 0.4	27.4 ± 0.2	23.5 ± 0.3	24.8 ± 0.3	24.9 ± 0.1	22.7 ± 0.5	14.5	9.4 ± 1.8	17.6 ± 3.4
18:1(n-7)c	4.9 ± 0.1	5.9 ± 0.1	4.7 ± 0.0	5.8 ± 0.1	5.6 ± 0.1	4.0 ± 0.0	4.5 ± 0.1	3.9	4.1 ± 1.0	3.9 ± 0.5
18:0	8.3 ± 0.0	7.4 ± 0.1	7.2 ± 0.1	8.5 ± 0.2	8.3 ± 0.1	7.5 ± 0.1	8.7 ± 0.1	13.3	10.9 ± 1.2	11.6 ± 1.0
20:4(n-6)	5.1 ± 0.1	2.4 ± 0.1	2.0 ± 0.0	2.6 ± 0.1	4.7 ± 0.2	4.3 ± 0.1	4.3 ± 0.1	6.1	5.2 ± 0.8	5.4 ± 0.3
20:5(n-3)	21.0 ± 0.2	10.1 ± 0.4	6.9 ± 0.1	8.2 ± 0.2	10.8 ± 0.1	8.8 ± 0.3	8.6 ± 0.2	10.2	14.1 ± 2.4	8.7 ± 0.5
20:2(n-6)	1.7 ± 0.0	0.0 ± 0.0	–	1.5 ± 1.3	0.0 ± 0.0	0.8 ± 1.5	1.8 ± 1.6	–	2.6 ± 0.2	2.1 ± 1.8
20:1(n-11)c	0.3 ± 0.0	1.7 ± 0.1	2.3 ± 0.0	0.8 ± 1.3	1.8 ± 0.0	1.7 ± 1.5	0.8 ± 1.4	–	–	1.1 ± 1.9
22:6(n-3)	13.5 ± 0.3	5.3 ± 0.3	4.0 ± 0.1	3.5 ± 0.1	5.4 ± 0.4	6.3 ± 0.2	4.9 ± 0.2	5.6	8.6 ± 1.5	5.9 ± 0.2
22:1(n-9)	0.8 ± 0.1	0.1 ± 0.0	1.6 ± 0.2	1.8 ± 0.3	0.1 ± 0.0	1.7 ± 0.2	1.9 ± 0.1	0.5	3.9 ± 2.6	3.2 ± 1.8
Other	17.5	9.0	9.7	10.3	8.6	9.2	10.8	14.0	17.1	13.4
Sum SFA	27.6 ± 0.3	22.2 ± 0.3	21.1 ± 0.2	24.6 ± 0.4	22.8 ± 0.1	20.1 ± 0.3	23.7 ± 0.9	35.6	34.8 ± 5.0	29.9 ± 3.4
Sum MUFA	23.8 ± 0.3	40.2 ± 0.4	40.8 ± 0.1	38.0 ± 1.0	37.0 ± 0.4	35.8 ± 1.4	34.4 ± 2.0	24.8	23.1 ± 0.7	29.8 ± 2.0
Sum PUFA	44.4 ± 0.7	37.3 ± 0.7	37.9 ± 0.2	35.7 ± 0.3	39.8 ± 0.6	43.0 ± 0.4	39.8 ± 0.8	39.6	37.6 ± 4.8	37.5 ± 1.6
Sum (n-3)	36.6 ± 0.5	16.5 ± 0.8	11.8 ± 0.2	12.4 ± 0.1	16.9 ± 0.5	15.9 ± 0.5	14.3 ± 0.3	18.3	24.0 ± 4.0	15.6 ± 0.3
Sum (n-6)	8.5 ± 0.1	20.4 ± 0.3	25.4 ± 0.3	24.1 ± 1.4	22.5 ± 0.1	27.2 ± 1.6	26.8 ± 1.9	21.3	15.2 ± 1.3	23.2 ± 2.6
Ratio (n-3)/(n-6)	4.3	0.8	0.5	0.5	0.8	0.6	0.5	0.9	1.6	0.7
Ratio EPA/AA	4.1	4.3	3.5	3.1	2.3	2.0	2.0	1.7	2.7	1.6
Ratio DHA/EPA	0.6	0.5	0.6	0.4	0.5	0.7	0.6	0.6	0.6	0.7

Presented as mean ± SD, $n = 3$; [a] Enriched *Artemia*; [b] Feed station (molted from stage II to III only); [3] $n = 1$; (–), below detection, AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; (–), below detection; Other includes components present at <2%: 14:0, i15:0, a15:0, 15:0, i16:0, C_{16} PUFA, 16:1(n-9)c, 16:1(n-7)t/16:2, 16:1(n-5)c, 16:0 Falde (fatty aldehyde), i17:0, a17:0, 17:1, 17:0, 18:3(n-6), 18:4(n-3), i18:0, 18:1(n-7)t, 18:1(n-5)c, 18:0 Falde, i19:0, 19:1, 20:3(n-6), 20:4(n-3), 20:1(n-11)c, 20:1(n-7)c, C_{21} PUFA, 21:0, 22:4(n-6), 22:5(n-3), 22:5(n-6), 22:1(n-11), 22:1(n-7), 24:1, 24:0.

Artemia, there was a concurrent drop in levels of AA (5 to 4%, 4 to 3 mg g^{-1} dry mass), EPA (21 to 9%, 14 to 6 mg g^{-1}) and DHA (14 to 5%, 9 to 3 mg g^{-1}), with a similar, although more pronounced, decrease in animals fed A1 DHA Selco–*C. muelleri*-enriched *Artemia* (AA: 3%, 2 mg g^{-1} dry mass; EPA: 8%, 5 mg g^{-1}; DHA: 4%, 2 mg g^{-1}). Conversely, levels increased in 18:1(n-9)c (8 to 23–24%) and 18:2(n-6) (1 to 18%). The FA profile of animals fed the Mussel powder–polar lipid diet closely reflected the diet, being dominated by 16:0 (12–15%), EPA (8–14%), DHA.(6–9%) and AA (5–6%). Compared to *Artemia*-fed phyllosomata, levels in the Mussel powder–polar lipid-fed animals of 18:1(n-9)c (9–18%) and 18:2(n-6) (5–13%) were lower, while in stages III and IV Mussel powder–polar lipid-fed animals, levels of 20:2(n-6) (2–3%) and 22:1(n-9) (3–4%) were higher.

DISCUSSION

A major feature of previous Australian feeding trials with southern rock lobster phyllosoma has been comparatively poor survival. This trial, however, represents a turning point in Australian rock lobster phyllosomata nutritional research, with greater than 80% survival of *Artemia*-fed phyllosomata through each stage from newly-hatched to stage V. We believe that a primary difference between this and the majority of previous trials has been the daily use of antibiotics in static culture. Although static culture and antibiotics are less appropriate for medium to large scale culture of phyllosomata (Ritar, 2001), they have been used in raising phyllosomata to pueruli (Matsuda & Yamakawa, 2000). Additionally, the growth results from this trial, although similar to a previous trial, had much tighter standard deviations (Nelson *et al.*, 2003b). This suggests that larvae from this trial had more similar environmental parameters resulting from both aquarium design and use of antibiotics. The successful use of antibiotics in static culture in this experiment highlights the fact that because the vital aspects of phyllosomata culture (i.e., feeding capabilities, nutritional requirements, aquarium design and microbial loading) are intrinsically linked, advances cannot be readily made sequentially, and should ideally be done concurrently. In prior trials, it has been difficult to test the effectiveness of feeding phyllosomata on *Artemia*, including using different enrichments, when experiments may be confounded by the adverse effects of microbial loading and aquarium design.

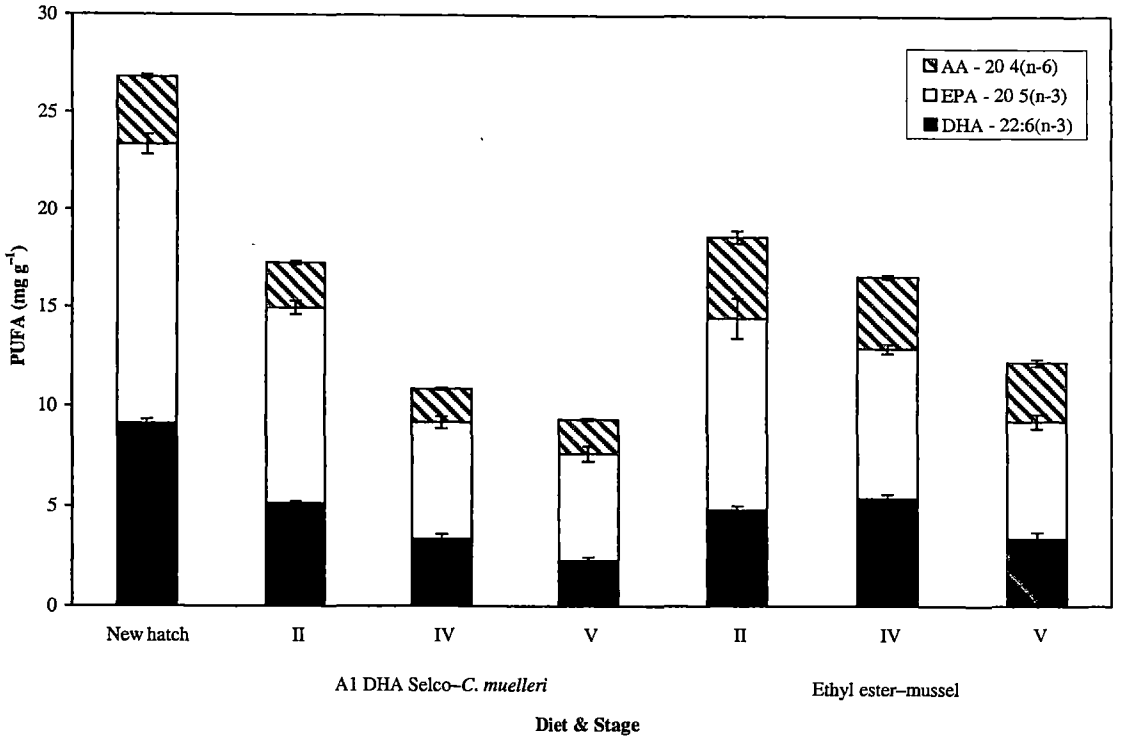

Figure. 2. Content (mg g^{-1}) of the essential long chain-polyunsaturated fatty acids, arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in *Jasus edwardsii* phyllosomata from stages I to V on two diet treatments of *Artemia* enriched with either A1 DHA Selco–*C. muelleri* or Ethyl ester–mussel nutrient sources. Presented as mean ± SD

The higher survival and good growth in this trial suggest that enriched *Artemia* may be adequate for early stage phyllosomata.

The dominance of TAG in enriched *Artemia* illustrates the propensity for readily incorporating TAG from nutrient sources, as well as metabolizing EE to TAG for assimilation into their tissues. Similar results were observed when providing *Artemia* with a high PL diet (Nelson, unpublished). These lipid class results are comparable to previous trials using 5-day old *Artemia* (Nelson *et al.*, 2002b; Smith *et al.*, 2002a; Nelson *et al.*, 2003b).

A distinction between this trial and an earlier trial (Nelson *et al.*, 2003b) is the detection of TAG in phyllosomata, albeit at low amounts, and the higher relative proportion of DG. Although the difference is small, the presence of these short-term energy storage molecules are consistent with improved larval health. In a prior feeding trial, total lipid content dropped markedly in phyllosoma to below 100 mg g^{-1} by stage IV, and was also accompanied by poorer survival (Nelson *et al.*, 2003b). This result contributed to the hypothesis that phyllosoma, like puerulus (Jeffs *et al.*, 2001a), may be better served by use of PL, rather than TAG (Nichols *et al.*, 2001;

Nelson *et al.*, 2003b). Animals in the present trial did not experience the same marked decrease in lipid content. This finding may be due to a number of reasons. Firstly, if lipid is critical to survival, a drop in total lipid is associated with the poorer survival in previous trials. Maintenance of lipid at above 100 mg g^{-1} in the present trial may therefore be linked with good survival. Secondly, since animals had high survival, but still did not have total lipid equal to wild phyllosomata (250 mg g^{-1} lipid dry mass at stage V) (Phleger *et al.*, 2001), the class of lipid provided (i.e., TAG in feeding trial versus largely PL in wild) was less effective. Thirdly, aquarium design and microbial loading can affect metabolism of lipids in larvae. For example, in previous trials, conducted in flow-through aquaria without antibiotics, and the present trial, conducted in static aquaria with antibiotics, *Artemia* that were similarly enriched with DHA Selco–*C. muelleri* were fed to phyllosomata. Animals from the present trial had 189 mg g^{-1} lipid dry mass at stage V, while animals in the previous trial had 50 mg g^{-1} lipid dry mass at stage V (Nelson *et al.*, 2003b). The animals in the previous trial either did not store lipid, or utilized more lipid as energy, while under the strain of microbes and/or swimming. Although *Artemia* supported excellent survival for larval stages I–V in the present trial, the *Artemia* diet may still not sufficiently condition phyllosomata for later stages; *Artemia* may not be providing adequate total lipid for growth and high survival, especially if the aspects of aquarium design and microbial loading are not addressed.

The current emphasis in phyllosoma culture in Australia is the use of *Artemia* for feeding stages I–V. This concept stems from developments with other aquaculture species, such as marine finfish, where it has been impossible to grow them during the early part of their life cycle without using live, motile feed (Olsen, 1997; Castell *et al.*, 1998). With rock lobster, complete rearing of phyllosoma to puerulus was achieved by feeding on *Artemia*, fish larvae and/or mussel tissue (Kittaka, 1997a, b; Kittaka & Abrunhosa, 1997; Matsuda & Yamakawa, 2000). Mussel gonad has been identified as the key to this success (Kittaka, 1997b), used exclusively after the third instar (Kittaka, 1997a). In culture, phyllosomata have been observed ingesting inanimate food particles, such as lobster, prawn and mussel pieces at late stages (Thomas, unpublished). Early stage animals have likewise been observed consuming pieces of mussel, jellyfish and other inanimate foods (Mitchell, 1971; Nelson *et al.*, 2002a; Cox & Johnston, 2003c). Phyllosomata in the present study were no excep-

tion. The larvae were observed consuming the Mussel powder–polar lipid feed station diet, a diet with which we attempted to build on the success of using mussel gonad. Additional evidence of feeding was the presence of faecal trails, and molting, considering that phyllosomata do not molt when not feeding (Abrunhosa & Kittaka, 1997c). Nevertheless, since phyllosomata fed the Mussel powder–polar lipid diet failed to molt properly beyond more than one stage, there is perhaps a necessary component either not present in sufficient amounts, or lost by leaching, in the feed station diet that contributes to molting. Therefore, the use of *Artemia* up to the third instar (Kittaka, 1997a) remains valuable for phyllosomata. However, to improve conditioning of larvae, the potential use of co-feeding of *Artemia* (Dhert *et al.*, 1999), along with a PL source, should be examined, particularly for later stage animals.

Of note is the decrease in essential PUFA from newly-hatched to stage V phyllosomata. On a relative basis, *Artemia*-fed phyllosomata and wild-caught animals at stage V had similar levels of AA (trial, 3–4%; wild, 2–3%) and EPA (trial, 8–9%; wild, 7–9%), with markedly lower DHA in cultured animals (trial, 4–5%; wild, 16–17%) (Phleger *et al.*, 2001). Results from a previous feeding trial are similar for relative levels of these FA (3–6% AA; 8–9% EPA; 2–4% DHA) (Nelson *et al.*, 2003b). However, since larval lipid remained above 189 mg g^{-1} lipid dry mass at stage V, on an absolute basis this trial represents a marked improvement for incorporation of essential PUFA. The fact that the amount of total lipid remained the same to stage V, but there was a drop in the level of essential PUFA, in particular DHA, highlights the importance of these FA. Higher absolute concentrations of these FA may be associated with enhanced survival and growth in this feeding trial compared with previous trials (Nelson *et al.*, 2003b); Hart *et al.*, unpublished). Total lipid and levels of essential PUFA in phyllosomata fed Ethyl ester–mussel-enriched *Artemia* were higher than in larvae fed A1 DHA Selco–*C. muelleri*-enriched *Artemia*. Since there was no direct association of enhanced FA profiles with survival and growth for phyllosomata from the two *Artemia* diet treatments, and the majority of lipid provided to phyllosomata through enriched *Artemia* was TAG, we propose that the improved survival and growth in this trial may result from the presence of lipid in the diet (as described above) in combination with better health. Furthermore, we suggest it is likely that the PUFA profiles will have a more significant effect if provided in a PL form.

These results also support the suggestion that co-feeding of *Artemia* and a PL source should be trialed to improve larval condition.

In conclusion, the use of antibiotics and static culture has enabled a clearer picture of the effects of nutrition on larval health. Our experiment demonstrated that lipid-enriched *Artemia* support excellent growth and survival in early stages of phyllosomata, and we are now better placed to take nutrition of phyllosoma forward. The results suggest that the class of lipid provided via *Artemia* may not adequately condition larvae, nor supply sufficient quantities of the essential PUFA, in particular DHA, for later stages. Thus, for successful culture of phyllosomata, the development of a formulated diet, which can provide the nutritional requirements in the right form to enhance long term conditioning of the larvae, is likely to be vital, particularly for later stage larvae.

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Chapter Six

Conclusions

If clarification is the objective, then focused research is the key. Research described in this thesis endeavors to identify key lipid nutrients vital to improving growth and survivorship of cultured Australian rock lobster (RL). In doing so, the thesis has provided insight into physiology, behavior and lipid trophodynamics applicable to many RL species. Through examination of wild *Jasus edwardsii* and *Jasus verreauxi* phyllosomata and pueruli, development of *Artemia* enrichment protocols, examination of phyllosoma feeding capabilities, and phyllosoma feeding trials utilizing enriched *Artemia* and feed stations, the acquired evidence indicates a physiological requirement of phyllosoma for high docosahexaenoic acid (DHA) lipid in polar lipid (PL) form. The observations of phyllosomata consuming food items suggests a range of potential prey items, further limited by the inability to process soft tissue. Interactions with environmental factors and effects of lipids in both diet and metabolism requires further study, however, this thesis will serve to supplement our growing knowledge, particularly concerning species which require PL. The outcomes of the research is encapsulated below, complimented by suggestions for the continued understanding of RL and consequently successful aquaculture.

Summary

The lipid and fatty acid (FA) profiles confirm the possibility of designing relatively simple and low-cost *Artemia* diets with polyunsaturated fatty acid (PUFA) profiles that may offer potential for use with RL larvae. The PUFA profiles of later-stage *J. edwardsii* phyllosomata, obtained here for the first time, change markedly during development. High levels of PUFA were obtained with *Artemia* fed simple diet mixes based on off-the-shelf oil products and other ingredients. Also, for the first time, *Artemia* were simultaneously enriched with all three essential PUFA, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and in particular arachidonic acid (AA). These enrichment results will be of potential use for aquaculture, more specifically larviculture, of *J. edwardsii*, particularly in earlier stages.

The results of *J. verreauxi* pueruli lipid analyses suggest that PL are their major energy store. Additionally, levels of essential PUFA, including linoleic acid, EPA,

DHA and AA are depleted in first instar juvenile RL following an extended period of non-feeding associated with swimming activity and metamorphosis from puerulus. This energy deficit may be responsible for the high mortalities of first instar juveniles that are commonly experienced in aquaculture and also probably in the wild. Supplying diets to first instar juveniles that are rich in these essential PUFA, preferably in PL, is likely to result in increased survival and growth of *J. verreauxi* and other RL species in aquaculture.

The results of a bead trial designed to indicate whether *Artemia* require the intake of particulate matter to enable gut evacuation, demonstrated that the gut content did not make a significant contribution to the total lipid content or FA profiles of juvenile *Artemia*. Therefore, any loss of gut content in juvenile *Artemia* as a result of feeding by phyllosoma was negligible. The short-term enrichment trial with juvenile *Artemia*, a life stage seldom used in feeding regimes, demonstrated the ability to assume the PUFA profile of their dietary source, in particular AA and EPA, and to a lesser degree, DHA.

Results of a long-term enrichment trial enabled formulation of a diet, which when fed to juvenile *Artemia*, resulted in a long-chain PUFA profile similar to wild early stage *J. edwardsii* phyllosomata. This enrichment was superior to other diets in terms of DHA incorporation. Using juvenile *Artemia*, we demonstrated the ability to easily alter the percentage of AA, which is particularly useful as AA has been identified as vital to nutrition in larval crustaceans. Lastly, this research identified the limitation of further increasing the percentage of DHA enrichment, an increase that would be useful, as levels in wild phyllosomata and potential prey items were markedly higher.

Examination of the lipid class (LC) and FA composition of cultured *J. edwardsii* phyllosomata fed with triacylglycerol (TAG)-enriched *Artemia* has provided insight into formulating future enrichment diets designed for RL phyllosomata. This thesis proposes that an appropriate diet should contain PL oil with a high percentage of DHA. Such an approach could increase delivery of the key essential PUFA.

After observing the feeding capabilities of *J. verreauxi* phyllosomata, this thesis suggests that neither jellyfish nor mussel gonad are suitable feeds for phyllosomata in aquaculture as they unduly foul larval appendages. Moreover, use of *Artemia* and mussel flesh is also not optimal given that the aim in culture water is to reduce

bacterial load. One can, however, aspire to formulate a feed with the more positive qualities of mussel, such as soft but stable texture and high nutritional levels. An appropriate formulated feed should be relatively inexpensive, low in microbes, firm, fleshy, and in discrete pieces that are not sticky or adherent; a diet that can be readily caught, manipulated, and ingested by RL phyllosomata.

The use of antibiotics in static culture with *J. edwardsii* phyllosomata has enabled a clearer picture of the effects of nutrition on larval health. The experiment demonstrated that lipid-enriched *Artemia* support high growth and survival in early stages of phyllosomata, and nutrition of phyllosoma can now more realistically be taken forward. The results suggest that the class of lipid provided via *Artemia* may not adequately condition larvae, nor supply sufficient quantities of the essential PUFA, in particular DHA, for later stages. Thus, for successful culture of phyllosomata, the development of a formulated diet, which can provide the nutritional requirements in the right form to enhance long term conditioning of the larvae, is likely to be vital.

In a companion study performed with *Panulirus cygnus*, PL was the main LC used during starvation and accounted for almost the entire increase in lipid in fed larvae. *Artemia* had high levels of TAG, however phyllosomata never accumulated TAG, suggesting phyllosomata are unable to absorb or accumulate TAG. The FA changes occurring in larvae appeared to be stage dependant, however PUFA, such as AA, EPA and DHA, were generally conserved. The results provide an excellent interspecies comparison and will assist in formulating diets for phyllosomata culture.

Future Research

This thesis has identified several vital aspects of culture of RL phyllosomata. Although not unique to phyllosomata, they must be resolved in a manner appropriate to the species. These include further exploration of feeding capabilities of phyllosomata, determination of the requirements for additional nutritional elements, suitable aquarium design, minimal microbial loading and optimal exposure of animals to a food source. This research specifically attempted to determine the nutritional requirements and feeding capabilities of phyllosomata. However, all aspects require ample investigation before additional advances in nutrition can be made. Further research should aim to refine the format of the feed station diet and identify the best

stage from when feed stations may be used, identify triggers for molting of phyllosoma and endeavor to reduce microbial loading in culture. A need still exists to test the feeding capabilities of phyllosomata with, and the preference for, locally available zooplankton. This thesis is not alone in demonstrating that *Artemia* "figure prominently, perhaps too prominently, in marine larvae production" and are unsuitable for supplying PL and high DHA (Sargent *et al.*, 2002), especially since *Artemia* are highly active in retroconverting DHA to EPA (Barclay & Zeller, 1996; Navarro *et al.*, 1999). For aquaculture of early-stage phyllosomata, copepods, although more adept at predator avoidance, may be a better alternative, as they provide levels, previously unmatched by *Artemia*, of essential PUFA through simple microalgal enrichment (Nanton & Castell, 1998; Stottrup, 2000). Alternatively, selection of specific *Artemia* strains with higher PUFA levels, and optimizing feeding regimes of *Artemia* can be examined. Later-stage phyllosomata may benefit by co-feeding of live feed and use of feed stations, a diet that avoids the often unpredictable metabolic complications of using live feeds, and may enable high DHA in PL form. Potential sources of oil having these characteristics include mussel and squid (Nichols *et al.*, 1998c), with an appropriate mussel extract trialed in Chapter 5. Additional research should aim to separate individual classes of PL for examination of PUFA, while keeping in mind the possibility that proteins may be an energy source, for further clarification of physiological requirements. Expansion of concepts examined in this thesis will prove a wise investment into clarification of the life history of RL and other crustacean species.

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Appendix One

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Lipids and nutrition of the southern rock lobster, *Jasus edwardsii*, from hatch to puerulus

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Abstract. We examined the lipid class and fatty acid composition of the southern rock lobster, *Jasus edwardsii,* phyllosoma larvae and puerulus stage to improve understanding of their nutrition in relation to aquaculture. Lipid is critical in the nutrition of larval crustaceans, including lobsters. Specimens were from Tasmanian waters, Australia, and North Island, New Zealand, waters. Analyses were by TLC-FID and capillary GC and GC-MS. Phyllosoma larvae and nektonic pueruli were low in storage lipid (triacylglycerol), and phospholipid was the major lipid class. Sterol, mainly cholesterol, was the next most abundant class. The ratio of the essential omega-3 fatty acid eicosapentaenoic acid (EPA) to the omega-6 fatty acid arachidonic acid (AA) was lower in newly hatched phyllosomata (1.2–1.3) than in other phyllosomata (stages III–XI; 2.8–6.7) and pueruli (3.8). Ratios of the omega-3 fatty acid DHA (docosahexaenoic acid) to EPA were also lower in newly hatched phyllosomata (0.5) than in later-stage phyllosomata (1.5–2.1) and pueruli (1.2). We have followed up these compositional data by successfully enriching the live diet (*Artemia*) of early phyllosomata with AA, EPA and DHA. This dietary manipulation has achieved ratios of these key polyunsaturated fatty acids similar to those of wild phyllosomata. These findings will be of significance to the future of rock-lobster aquaculture.

Extra keywords: phyllosoma, phospholipid, fatty acids, polyunsaturated fatty acids, EPA, DHA, AA, sterol, diacylglycerol

Introduction

The Australian rock-lobster fishery is an important natural resource making up 25% of the value of Australia's total fishery landings and presently worth around A$450 million per annum. The fishery for southern rock lobster, *Jasus edwardsii*, is the second most valuable fishery in Tasmania, having a value of approximately A$40 million (Punt & Kennedy, 1997). This lobster species is also one of the most valuable fisheries in New Zealand, involving annual exports of over NZ$100 million (Breen & Kendrick, 1997). However, most rock lobster fisheries are fully exploited, so future expansion of output can only occur through aquaculture and enhancement programs.

The southern rock lobster goes through 11 planktonic larval stages lasting from 12 to 24 months before metamorphosis to the benthic puerulus stage (Lesser, 1978; Phillips & Sastry, 1980; Booth, 1994). Some progress has been made in rearing *J. edwardsii* larvae to puerulus, notably by Kittaka (1994a; 1994b). More recently, Illingworth *et al.* (1997) obtained mean survival rates of over 60% to stage VII, but as very little is known about feeding in the wild, a need clearly remains for further nutritional research on this species. Larval nutrition is deemed a critical issue.

Lipids are very important in animal nutrition and, for crustaceans in particular, are critical for certain larval stages. There are, however, very few studies of lipids and fatty acids for any rock-lobster species. Essential fatty acids, such as eicosapentaenoic acid [EPA, 20:5 (n-3)] and docosahexaenoic acid [DHA, 22:6 (n-3)], cannot be produced *de novo* in most crustacean species in sufficient quantities to allow normal metabolic functioning, so they must be obtained from the diet (Kanazawa *et al.*, 1979). The fatty acid composition of newly hatched phyllosomata was found to be high in arachidonic acid [AA, 20:4 (n-6)], which may be correlated with maternal diet (Smith, 1999), but to date no published studies have addressed the lipid class and fatty acid composition of later-stage phyllosomata of *J. edwardsii*. Lipid is the primary energy-storage component of the puerulus of *J. edwardsii* and allows it to swim from the open ocean to shallow coastal waters (Jeffs *et al.*, 1999). Phospholipid is the major lipid class of the puerulus and supplies energy for crossing the continental shelf of New Zealand (Jeffs *et al.*, 2001a). The two essential fatty acids EPA and DHA increased at the first-instar juvenile stage of *J. edwardsii* (Pearce, 1997), and the increase may be correlated with commencement of feeding (Pearce, 1997). Lipid class and fatty acid profiles are available for commercially harvested adult *J. edwardsii* (Nichols *et al.*, 1998c).

The major aim of our study, therefore, was to determine the signature lipid profiles of later-stage phyllosomata of *J. edwardsii*. These data are essential to providing a nutritionally balanced diet for susceptible larval stages in culture. A second aim was to assess the effect of preservation methods on lipid class and fatty acid composition. We undertook this task to examine the feasibility of analyzing preserved larval samples; if possible, analysis of the lipid composition of archived larval samples would enable aquaculturists to manipulate diets more effectively to provide the correct lipid composition to larvae. Enrichment studies using essential fatty acids such as EPA and DHA have been successfully carried out on the rotifer *Brachionus plicatilis* (e.g., by Nichols *et al.*, 1989; Lewis *et al.*, 1998). Enrichment studies with the brine shrimp *Artemia salina* have been conducted with omega-3 essential fatty acids to improve larval fish culture (Watanabe *et al.*, 1982; Evjemo *et al.*, 1997). Unless enriched, *Artemia* fail to provide a nutritionally balanced dietary food source with respect to essential fatty acids. Most techniques for newly hatched *Artemia salina* have been developed to suit the requirements of larval fish and prawns (Rees *et al.*, 1994; McEvoy & Sargent, 1998). Therefore our third aim was to manipulate the AA, EPA, and DHA composition of *Artemia* over a 5-day grow-out cycle to suit the requirements of southern rock lobster phyllosomata.

Materials and methods

Rock lobster preservation experiment

Two ovigerous females (approximately 600 g each), caught in coastal waters off southern Tasmania, were held for two months (September and October 1999) at TAFI Marine Research Laboratories, Hobart. Eggs hatched on 3 November 1999 and subsamples of pooled newly hatched phyllosomata (300–400 individuals, 0.3–0.4 g wet mass) were immediately preserved in 100% ethanol or 10% formalin in either glass or polypropylene jars. Pooled newly hatched phyllosomata were also either extracted fresh in chloroform/methanol/water, fresh frozen at –20°C, or lyophilized.

Wild rock lobster larvae—ethanol samples

Existing phyllosoma and puerulus samples of *Jasus edwardsii* preserved in 100% ethanol were obtained from Russell Bradford at CSIRO Marine Laboratories. These samples included stage III–V and VII phyllosomata and nektonic pueruli and were mostly collected from RV *Southern Surveyor* between 12 February and 4 March 1998 at between 34°68'–41°78'S and 133°36'–144°45'E (off the west coast of Tasmania). One phyllosoma sample (stage V) was collected from RV *Franklin* during March 1997 between 43°68–44°15'S and

145°40'–146°84'E. Six net systems were routinely used to collect these samples (Bruce *et al.*, 1996). Samples were preserved in ethanol within 10–20 min of being hauled aboard. Most samples were from surface tows (upper 2 m) conducted during the night, and they were subsequently stored in the dark at room temperature.

Wild New Zealand phyllosoma samples

Wild *Jasus edwardsii* phyllosomata (stages V–IX, XI) were collected from RV *Tangaroa* by Engel net (fine-meshed midwater trawl) towed at a depth of 30 m at less than 2 knots for 1 h. The trawl was taken at 39°9.9'S, 178°41.4'E (2250–2360 m) 56 km off the coast of the North Island, New Zealand, on 12 April 2000 at night (2200–2300). All phyllosomata were sorted from the rest of the catch upon net landing and transferred into liquid nitrogen while alive. The frozen samples were shipped to CSIRO Marine Laboratories and analyzed immediately. Fresh masses were 0.2718 g for stage XI phyllosomata (1 only), 0.0877 g for stage IX (1 only), 0.04982 g for stage VIII (5 individuals pooled), 0.3473 g for stage VII (5 pooled), 0.2127 g for stage VI (5 pooled), and 0.2227 g for stage V (5 pooled). For comparative purposes, 10 L of seawater collected by CTD 3 m below the surface was filtered for phytoplankton and other particulate matter at the area where the New Zealand phyllosomata were collected.

Feeding experiment—Artemia enrichment

Artemia cysts (INVE, Great Salt Lake Prime Gold) were decapsulated and hatched at 28 ± 1°C in 50-L white fibreglass cones in filtered brackish water (0.2 μm, 27 ± 1‰). After 24 h, newly hatched *Artemia* nauplii were removed from the hatching cones, rinsed in fresh water for 2 min, and transferred to 1000-L conical tanks at a density of 4 animals ml^{-1} in filtered seawater (0.2 μm, 34 ± 1‰, 27 ± 1°C). *Artemia* were fed twice daily with an oat-bran-based diet consisting of oat bran, wheat germ, and lecithin (50:6:4 by weight).

After 4 days, *Artemia* were harvested, rinsed, suspended (10,000 *Artemia*) in 1-L beakers containing seawater and fed one of three enrichment diets (see below) for 24 h. Triplicate samples (10,000 *Artemia*) for each enrichment were rinsed and stored for analysis. The three enrichment diets were added to beakers at a rate of 0.6 g L^{-1} and consisted (by weight) of (1) *Tetraselmis suecica*, cultured in batches, cell density measured daily; (2) oat bran, wheat germ, and lecithin (50:6:4), plus MaxEPA and A1 ARASCO (arachidonic acid single cell oil) (40% in 4.5:1 ratio; Martek Biosgenes, USA) (for convenience, we have termed this diet oats/EPA/AA); and (3) A1 DHA-Selco (INVE Group, Belgium).

The diets were prepared daily; each was blended finely in 500 ml of fresh water, passed through a 63-μm screen, and transferred to the beakers. The fibrous nature of oats prevented all of the diet from passing through the screen, so the remaining portion was dried and weighed for calculation of the daily amount fed. The performance of each diet was assessed in terms of survival, growth, and fatty acid and lipid profiles of *Artemia*.

Sample preparation

Artemia and feed samples were filtered through 4.7-cm Whatman glass filters (GF/F) and rinsed with 0.5 M ammonium formate. They were lyophilized overnight before analysis. Dry masses of the samples ranged from 56 to 263 mg, and masses of lipid were 0.8–49.7 mg.

Lipid extraction and analysis

Samples were quantitatively extracted with a modified Bligh and Dyer (1959) one-phase methanol:chloroform:water extraction (2:1:0.8, by vol); the samples were extracted overnight, and the phases were separated the following day by the addition of chloroform and water (final solvent ratio, 1:1:0.9, by vol, methanol:chloroform:water). The total lipid was concentrated (i.e., solvents were removed *in vacuo*) by rotary evaporation at 40°C. Lipid class analyses were conducted immediately; samples were stored for no more than 3 days in a known volume of chloroform.

We analyzed an aliquot of the total lipid using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to determine the abundance of individual lipid classes (Volkman & Nichols, 1991). Samples were applied in duplicate or triplicate to silica-gel SIII Chromarods (5-μm particle size) with 1-μl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane:diethyl ether:acetic acid (60:17:0.2, by volume), a mobile phase resolving nonpolar compounds such as wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA), and sterols (ST). A second non-polar solvent system of hexane/diethyl ether (96:4 by volume) was also used for selected samples to separate hydrocarbon from WE and TAG from diacylglyceryl ether (DAGE). After development, the chromarods were oven-dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, WE [derived from fish oil], TAG [derived from fish oil], and DAGE [purified from shark liver oil]; 0.1–10 μg range). Peaks were quantified on an IBM-compatible computer with DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ±5–10% for individual lipid classes (Volkman & Nichols, 1991; Nichols *et al.*, 1998a).

An aliquot of the total lipid was treated with methanol:hydrochloric acid:chloroform (10:1:1, by volume; 80°C, 2 h). The fatty acid methyl esters (FAME) products were extracted into hexane:chloroform (4:1, by volume, 3 × 1.5 ml) and the FAME mixture was treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 50 μl, 60°C, 1 h) to convert sterols to their corresponding TMSi (trimethylsilyl) ethers.

Gas chromatographic (GC) analyses of methyl esters of fatty acids were performed with a Hewlett Packard 5890A GC (Avondale, PA, USA) equipped with an HP-1 cross-linked methyl silicone fused-silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector, and an HP 7673A auto sampler. Hydrogen was the carrier gas. After addition of methyl nonadecanoate and methyl tricosanoate internal standards, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1}, and finally to 300°C at 5°C min^{-1}. Peaks were quantified with Waters Millennium software (Milford, MA, USA). Individual components were identified from mass spectral data and by comparison of retention-time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component abundance. GC–mass spectrometric (GC–MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

Results

Lipid classes—newly hatched phyllosomata (preservation experiment) and adults

Phospholipid (PL) was the major lipid class in newly hatched phyllosomata and adults (Table 1). Lowest PL values occurred in formalin-preserved newly hatched phyllosomata. FFA were also somewhat higher in formalin-preserved newly hatched phyllosomata than in other samples. ST (mainly cholesterol) was the second most abundant lipid class. Minor levels of WE and very low levels of TAG were only present in newly hatched phyllosomata (Table 1). Minor levels of diacyglycerols (DG) were detected in newly hatched phyllosomata. Lipid was 43.5–56.5 mg g^{-1} dry mass in newly hatched phyllosomata and 31.0 mg g^{-1} dry mass in adults.

Lipid classes—wild later-stage phyllosomata

PL was the major lipid class in all *J. edwardsii* phyllosomata from both Tasmanian and New Zealand ocean waters (Table 1). DG was the second most abundant in all New Zealand phyllosomata but was only second in one Tasmanian phyllosoma sample (stage V) and equal

Table 1. Percentage lipid class composition of southern rock lobster, *Jasus edwardsii*.

	n	Wax esters	Triacyglycerols	Free fatty acids	Diacyglycerols	Sterols	Polar lipids	Lipid as mg g^{-1} dry mass
Adult[1]	3	–	–	0.2 ± 0.3	–	8 8 ± 0.6	91 ± 0 8	31.0 ± 4.0
Newly hatched phyllosoma - variation with storage treatment[3]								
Fresh	3	0 7 ± 0.1	0.2 ± 0.0	-	0.5 ± 0.1	6.6 ± 0.9	92.0 ± 1.1	54.5 ± 9.0[2]
Frozen	2	0.8 ± 0.1	0.3 ± 0.1	0 5 ± 0.4	0.5 ± 0 1	6.4 ± 0.0	91 5 ± 0.5	56.5 ± 2.5[2]
Lyophilized	2	0 7 ± 0.2	0 2 ± 0.1	0.8 ± 0.1	0.4 ± 0.0	6.2 ± 0.5	91.6 ± 0.9	45 0 ± 4.0[2]
Ethanol	2	1.0 ± 0.0	0.5 ± 0.0	2.0 ± 0.5	0.7 ± 0.1	6.4 ± 0.2	89 3 ± 0 7	49.0 ± 1 5[2]
Formalin	2	0.6 ± 0.0	0.3 ± 0.1	3.0 ± 0.0	0.2 ± 0 0	8.2 ± 0.7	87.6 ± 0.8	43.5 ± 1.5[2]
Phyllosoma - west coast Tasmania								
Stage IV	9[4]	–	–	–	5.5	12.0	82.5	107.4
IV, V	2	–	–	–	4.0	4.1	91.9	235.3
V	1	–	–	–	7.1	1.0	91.9	347.3
VI	1	–	–	–	2.0	2.0	95.9	94.6
VII	2	–	–	–	2.1	5 5	92.4	319.1
Phyllosoma - New Zealand								
Stage V	5	tr	0.2	0 1	6.2	1.5	91.9	206.6[2]
VI	5	–	0.3	0.1	4.5	0 8	94.3	242.1[2]
VII	5	–	0.1	0.1	6.9	1.4	91.4	194.4[2]
VIII	5	–	0.1	0.1	5.2	1.2	93.4	185.7[2]
IX	1	–	0.1	0.3	6.1	1.4	92 1	279.4[2]
XI	1	–	0.1	0.1	5.2	1.9	92.7	344.0[2]
Pueruli - west coast Tasmania								
Nektonic	3	–	–	–	4.1 ± 1.2	1.4 ± 1 0	94.5 ± 1.9	34.8 ± 17.9

presented as mean ± sd; –, below detection, tr, trace <0.05%; [1]from Nichols *et al* (1998b), [2]calculated from wet mass assuming 80% water, [3]storage treatments - all 90 days, fresh samples extracted at time of harvest, 300-400 newly hatched phyllosoma per sample; [4]pooled individuals.

to ST in stage VI Tasmanian phyllosomata. ST was the third major lipid class in New Zealand phyllosomata and was second in abundance in most Tasmanian phyllosomata. Cholesterol was the major sterol present. New Zealand phyllosomata also had very low TAG and FFA, but neither these lipid classes nor WE was detected in Tasmanian phyllosomata (Table 1). Lipid levels were 95–347 mg g^{-1} dry mass in Tasmanian phyllosomata and 186–344 mg g^{-1} in New Zealand phyllosomata.

When calculated as milligrams per phyllosoma, total lipid increased markedly at stages IX–XI for New Zealand phyllosomata (55.9–68.8 mg lipid per animal) compared to stages V–VIII (7.4–9.7 mg lipid per animal). PL was the major lipid class (71.9–80.6% of total lipid) in filtered phytoplankton and other particulate matter collected at the area where the New Zealand phyllosomata were collected; TAG was second (8.9–17.6%) and WE third in percent composition (6.4–12.3%). Lipid content of these particulate-matter samples was very low (0.01–0.02 mg L^{-1}).

Lipid classes—wild pueruli

Nektonic *J. edwardsii* pueruli from Tasmanian waters were dominated by PL and had low DG and ST (Table 1). Lipid levels were quite low compared to those of phyllosomata, 34.8 mg g^{-1} of dry mass. No WE, TAG, or FFA was detected.

Fatty acids—newly hatched phyllosomata (preservation experiment) and adults

The main fatty acids in all newly hatched phyllosomata were 16:0, 18:1(n-9)c, AA, EPA, and DHA (Table 2). AA comprised 11.6–11.9% (of total FA). Formalin-preserved newly hatched phyllosomata and adult *J. edwardsii* had less AA. AA values for the newly hatched phyllosomata were considerably higher than those for all other phyllosoma samples and higher than those for the puerulus (Table 2, Fig. 1). EPA values were also higher for newly hatched phyllosomata (frozen, lyophilized, and ethanol-preserved) and adults; formalin-preserved newly hatched phyllosomata showed lower values. EPA values were higher for

newly hatched than for later-stage phyllosomata samples and nektonic pueruli. The EPA/AA ratios were thus lowest in newly hatched phyllosomata (1.2–1.3), higher in adults (2.5), and highest in later-stage phyllosomata (2.8–6.7) and pueruli (3.8). DHA values were higher in adult southern rock lobsters (16.6% of total FA) than in newly hatched phyllosomata (less in formalin-preserved specimens, Table 2). Later-stage phyllosomata had higher DHA than newly hatched phyllosomata. Nektonic pueruli also had higher DHA (Table 2, Fig. 1). These differences are reflected in DHA/EPA ratios for adults (0.9), newly hatched phyllosomata (0.5), later-stage phyllosomata (1.5–2.1) and pueruli (1.2). Newly hatched phyllosomata had more DPA [docosapentaenoic acid, 22:5 (n-3)] than adult lobsters, later-stage phyllosomata, and pueruli (Table 2). The total polyunsaturated fatty acids (PUFA) for adults and newly hatched phyllosomata was also greater than for later-stage phyllosomata and pueruli.

The total monounsaturated fatty acids (MUFA) was also lower for adults and newly hatched phyllosomata. Oleic acid [OA, 18:1(n-9)c] values for adults and newly hatched phyllosomata were lower than those for other samples (Table 2). Vaccenic acid [18:1(n-7)c] levels were slightly higher for newly hatched phyllosomata than for other samples. There were lower levels of 20:1(n-11)c fatty acid in newly hatched phyllosomata than in later-stage phyllosomata and pueruli (Table 2). This fatty acid was not detected in adults.

The PUFA 18:4(n-3) was not detected in adults, newly hatched phyllosomata, or pueruli but was present at low levels in later-stage phyllosomata (Table 2). Branched fatty acids were present at less than 1% in all specimens and all stages. The major saturated fatty acid (SFA) in all specimens was palmitic acid (16:0); less was detected in adults and newly hatched phyllosomata (more for formalin-preserved samples) than in other samples (Table 2). This difference is reflected in the total SFA for adults and newly hatched phyllosomata (more for formalin-preserved specimens); less was detected than in later-stage phyllosomata and pueruli.

Fatty acids—wild later-stage phyllosomata

Values of AA were lower in later-stage phyllosomata than in newly hatched phyllosomata, and some locations differed slightly. Tasmanian phyllosomata had less AA than New Zealand phyllosomata (Table 2, Fig. 1). Some between-location differences in EPA, which was lower in later-stage phyllosomata than in newly hatched phyllosomata, were also evident. Tasmanian phyllosomata had less EPA than New Zealand phyllosomata. More DHA was detected in later-stage phyllosomata than in newly hatched phyllosomata, and less on average was found in Tasmanian phyllosomata. The PUFA 18:4(n-3) was lower in Tasmanian phyllosomata, and 18:4(n-3) also decreased slightly with increasing stage in New Zealand phyllosomata (Table 2). OA was higher in Tasmanian phyllosomata. It generally increased with increasing phyllosoma stage in New Zealand phyllosomata. The MUFA 20:1(n-11)c was also present at higher levels in later stages of the New Zealand phyllosomata.

The fatty acid composition of three particulate-matter samples from the New Zealand waters where the phyllosomata were collected showed 0.3% AA, 1.6–2.9% EPA, and 4.9–7.7% DHA (ratios of EPA to AA, 5.4–10.9; DHA to EPA, 2–3.5). The sum (n-3) was 10–15% (% of total FA) and the sum (n-6) was 4.3–4.8% [ratio (n-3)/(n-6) 2.2–3.5]. The sum SFA was 40–46%, the sum MUFA 28–33.6%, and the sum PUFA 14.9–19.3%.

Feeding experiment—lipid classes of feeds and Artemia

The algal feed contained mostly PL (Table 3). Oats/EPA/AA and DHA Selco feeds contained primarily TAG, with less PL and much less ST, FFA and WE. Lipid contents of the feeds only were 109 mg g^{-1} (algae, *T. suecica*), 288 mg g^{-1} (oats/EPA/AA) and 806 mg g^{-1} (mg g^{-1} dry mass) (DHA Selco). For *Artemia* fed these diets, lipid was 98 mg g^{-1} dry mass on the algal diet, 259 mg g^{-1} on the oats/EPA/AA diet, and 297 mg g^{-1} on the DHA Selco diet. *Artemia* fed the algal diet had primarily PL with less ST, FFA, TAG and WE (Table 3). *Artemia* fed either of the lipid-rich diets were high in TAG with PL second in abundance.

Table 2. Percentage fatty acid composition of southern rock lobster *Jasus edwardsii*.

	Adult	Newly hatched phyllosoma - variation with storage					Phyllosoma - TAS					Phyllosoma - NZ						Puerulı - TAS
		Fresh	Frozen	Lyophilized	Ethanol	Formalin	IV	IV, V	V	VI	VII	V	VI	VII	VIII	IX	XI	Nektonic
i14:0	2.1 ± 1 9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14·0	1.6 ± 0 1	0.8 ± 0.0	0.8 ± 0.0	0 8 ± 0 0	0 9 ± 0 0	1 1 ± 0.0	4.4	2 7	3.6	3.9	3.9	3 7	3.6	3.5	3 7	3 7	3.2	2 3 ± 0.5
15 0	1.1 ± 0.1	0.7 ± 0 0	0.7 ± 0.0	0 8 ± 0 0	0.7 ± 0 0	1 0 ± 0.0	1.1	1.2	1 6	1 2	1.8	1.3	1.4	1 4	1.4	1 5	1 0	0 7 ± 0 2
16·1(n-9)c	–	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0 0	0 1 ± 0.0	0 1 ± 0.0	2 6	2.4	0.0	2.2	1.9	tr	tr	tr	tr	tr	tr	0 3 ± 0.2
16.1(n-7)c	4.6 ± 0 3	3.3 ± 0.1	3 6 ± 0.2	3.7 ± 0.0	3.4 ± 0.0	4 4 ± 0.1	4.7	2.6	6.5	3 8	4.5	6.0	5.9	6.2	6.3	6.9	5 9	5.0 ± 0.6
16:1(n-7)t/16·2	–	0.3 ± 0 0	0 2 ± 0.1	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.6	0.4	1 5	0.3	1.5	1.1	1.2	1.3	1.1	1.2	0.8	0 7 ± 0.3
16:0	13.2 ± 0.7	14.5 ± 0 5	14.6 ± 0 1	14.5 ± 0.1	14.2 ± 0.1	18.2 ± 0.2	14.1	15.5	21.1	21.7	18.6	19 7	19 7	20.2	20.2	20.5	19.7	15 6 ± 2.6
i17:0	0.5 ± 0 1	0.7 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0 0	1.0 ± 0.0	0.1	0.5	0 2	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0 2	0.2 ± 0.1
a17:0	–	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0 6 ± 0 0	0.7 ± 0.0	–	–	–	–	–	0.3	0 3	0.3	0.3	0 3	0 2	–
17·1(n-8)c	1.6 ± 0.2	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0 0	0.6 ± 0.0	0.8 ± 0.0	1.3	1.5	0 9	0.9	1.1	0.5	0 6	0.6	0.6	0 6	0.5	0.8 ± 0.1
17:0	1.6 ± 0.1	1.5 ± 0.0	1.6 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.8 ± 0.0	0.7	1 0	0.7	0.7	1.1	1.0	1.0	1.1	1 0	1.0	0.8	0 8 ± 0.1
18:4(n-3)c	–	–	–	–	–	–	0.6	0.7	1.0	0.6	0.9	1 4	1.3	1.2	1.3	1.2	0.9	–
18:2(n-6)/a18:0	1.7 ± 0.2	1 4 ± 0.1	1.3 ± 0 0	1.3 ± 0 0	1.3 ± 0.0	1.1 ± 0 0	3.4	6.5	1.1	2.0	1.6	1 1	1 2	1.1	1.2	1.2	0 9	1.7 ± 0.7
18:1(n-9)c	12 2 ± 0.4	12.6 ± 0.2	13.3 ± 0.1	12.9 ± 0 2	12.7 ± 0.1	15.9 ± 0 2	30.6	26.7	19 1	24.6	21.0	16 6	16.1	17.1	17.2	17.1	20.0	26.5 ± 7.7
18:1(n-7)c	2.8 ± 0.5	5.1 ± 0.2	5.2 ± 0.1	5.2 ± 0 1	5.3 ± 0.1	6.5 ± 0 0	4 3	4.7	2 8	1 8	3.2	2.3	2.3	2.3	2 3	2.2	2.8	4.2 ± 0.8
18:0	6.6 ± 0.3	6.9 ± 0 2	6.9 ± 0 2	7.1 ± 0.1	7.0 ± 0.0	8.5 ± 0.1	6.2	8.5	7 7	10.5	7.6	7 8	8.1	7.9	8 0	8.0	8.4	9.1 ± 0.3
20:4(n-6) - AA	7.3 ± 0.7	11.6 ± 0 3	11.7 ± 0.2	11.9 ± 0.2	11.6 ± 0.1	8.4 ± 0 1	1.7	1.4	1 9	1.1	1.6	3.0	3.1	3.0	2.8	2 8	2.1	2.4 ± 0 2
20:5(n-3) - EPA	18.1 ± 1.1	15.1 ± 0.4	14 8 ± 0 0	14.9 ± 0.2	15.3 ± 0.3	10.3 ± 0 1	7.5	5 9	7.7	7.4	6.9	8.8	9.4	8.5	8.6	7.7	8.6	9 2 ± 0.8
20:2(n-6)	0.7 ± 0.0	2.1 ± 0 1	2.2 ± 0 0	2.1 ± 0 1	2.1 ± 0 0	2.4 ± 0.1	0.2	0 4	0.5	0.3	0.6	0.5	0.5	0.4	0 5	0.5	0.5	0 5 ± 0.1
20:1(n-11)c	–	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0 0	0 6 ± 0.0	0 7 ± 0 0	1.1	1.2	2 3	1.3	1.3	2.0	1.9	2.1	1.9	1.8	4 4	3.3 ± 1.1
20:1(n-9)c	2.8 ± 0 2	2.0 ± 0.3	1 8 ± 0.1	1 8 ± 0.0	1.9 ± 0 0	2.3 ± 0.1	0.1	0.3	0.5	0.2	0.4	–	–	–	–	–	–	0.6 ± 0.3
20:0	1 0 ± 0 1	0.6 ± 0 0	0.6 ± 0 1	0.6 ± 0.0	0.5 ± 0.0	0.7 ± 0.1	0.6	0.5	0.4	0.7	0.7	0.5	0.5	0.5	0.5	0.5	0.4	0.4 ± 0.1
22.6(n-3) - DHA	16 6 ± 1.3	8.1 ± 0.1	7.6 ± 0.1	7.8 ± 0 0	7.9 ± 0.1	4.7 ± 0.1	11.1	10.2	15.6	12.0	14 1	16.6	16.5	15.7	16.0	16.3	13.6	11.0 ± 2 6
22:4(n-6)	0.1 ± 0.2	1 5 ± 0.0	1.5 ± 0 1	1.5 ± 0 0	1.6 ± 0.0	1.1 ± 0.0	–	tr	0.1	–	1.8	0.1	0 1	0.1	0.1	0.1	0.1	0.1 ± 0.1
22:5(n-3) - DPA	0.7 ± 0.1	2.0 ± 0.0	1.9 ± 0.1	1.9 ± 0.0	2.0 ± 0.0	1.2 ± 0 0	0.2	0.3	0.6	–	0.2	0.6	0.5	0 5	0.5	0 5	0 4	0.4 ± 0.2
22:1(n-11)	0.6 ± 0.1	0 2 ± 0 1	0.2 ± 0.0	0.2 ± 0.0	0 2 ± 0.0	0.2 ± 0.0	–	–	–	–	0.7	tr	tr	tr	tr	tr	0 5	0 5 ± 0.3
22:1(n-9)	–	0.2 ± 0 1	0.2 ± 0.0	0 2 ± 0.0	0 2 ± 0 0	0.2 ± 0 1	1.0	3.1	0 5	0.8	1.2	0.2	0.1	0.2	0 2	0.1	0 5	1.0 ± 0.4
Other	2.7	6.9	6.7	6 4	6.6	6.0	2.0	1.8	2.3	1.7	0.8	4 6	4.5	4.8	4 2	3.9	3.7	2.9
Sum SFA	28.1 ± 1.3	28 2 ± 0.6	28.6 ± 0.2	28.8 ± 0 2	28.1 ± 0 1	35 1 ± 0 5	29.4	32.3	37.3	40.6	35.7	31.6	31.8	32.2	32.2	32.8	31.3	30 9 ± 3.9
Sum MUFA	25.4 ± 0 8	27.6 ± 1.0	27.5 ± 0 1	27.2 ± 0 0	27.1 ± 0.0	33.7 ± 0.2	46.8	43.1	34.1	36 3	37.4	30.1	29.4	30.8	30.8	31.2	36 7	43.3 ± 6.5
Sum PUFA	46.5 ± 1.0	43.5 ± 0.6	42.6 ± 0.2	43.1 ± 0.1	43.4 ± 0.2	30.7 ± 0.2	25 0	26.2	29.5	24.0	28.1	33.5	34 1	32.2	32.3	31.8	28.6	26 5 ± 2.8
Sum (n-3)	36.1 ± 1.8	25.6 ± 0.6	24.7 ± 0 1	25.0 ± 0 1	25.7 ± 0.3	16 6 ± 0.2	19 5	17.3	25.3	20.0	22.3	27.8	28.1	26.4	26.9	26 1	24.3	21.3 ± 3.4
Sum (n-6)	10.3 ± 0.9	17.2 ± 0.4	17.4 ± 0.1	17.5 ± 0.2	17.2 ± 0.1	13.7 ± 0 1	5.5	8 7	4.1	4.0	5.7	5.5	5.8	5.6	5.2	5 6	4.2	5.1 ± 0.7
Ratio (n-3)/(n-6)	3.50	1.49	1 42	1.43	1.49	1.22	3.53	1.98	6.10	5 02	3.88	5.04	4.86	4 67	5.18	4.68	5.81	4.14
Ratio EPA/AA	2.47	1.31	1.26	1.26	1.33	1.23	4.53	4.31	4.13	6.73	4.27	2.91	3 04	2.85	3.09	2.77	4 05	3.77
Ratio DHA/EPA	0.92	0.54	0.52	0.52	0.52	0.46	1.47	1.74	2.02	1.64	2.02	1.88	1 75	1.84	1.86	2.10	1.59	1.19

presented as mean ± sd; –, below detection; tr, trace <0 05%, AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid, DPA, docosapentaenoic acid; details provided in Table 1; Other includes components present at <1%· i15:0 a15:0, i16·0, C_{16} PUFA, 16:1(n-5)c, i18:0, 18·3(n-6)c, 18:3(n-3)c, 18:1(n-7)t, 18·1(n-5), i19 0, 19:1, 20.3(n-6), 20:4(n-3), 20:1(n-7)c, C_{21} PUFA, 21:0, 22:5(n-6), 22:3(n-3), 22·1(n-7), 22:0, 24·1, 24:0.

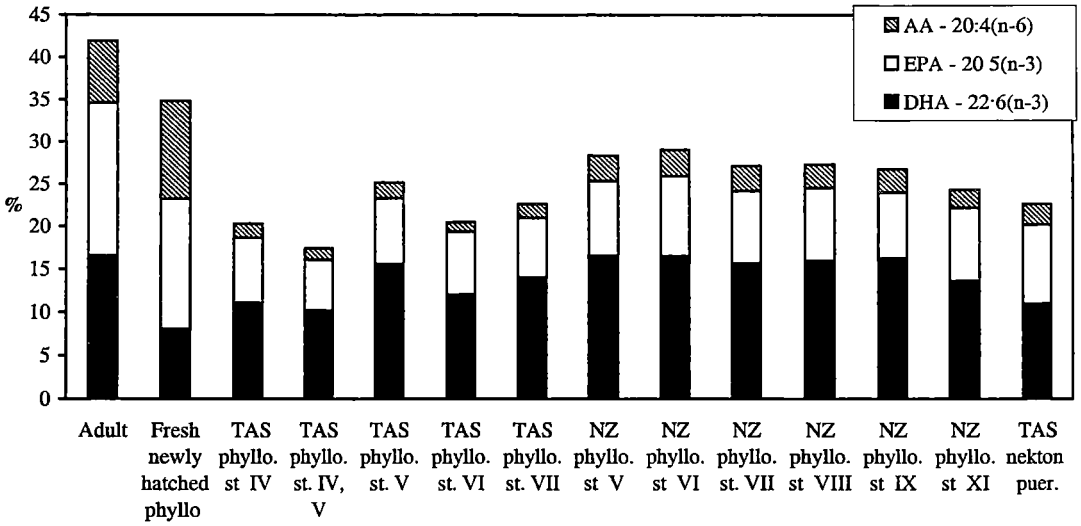

Fig. 1. Percent composition of arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in southern rock lobster, *Jasus edwardsii*. Phyllo = phyllosoma; puer. = pueruli.

Artemia feeds—fatty acid composition

The algal feed had as the major PUFA 18:2(n-6), 18:4(n-3), EPA, and C_{16} PUFA (Table 4). DHA was low in the algal feed, reflected in the very low DHA/EPA ratio (0.04). The EPA/AA ratio was 3.3. These PUFA were reflected in a high total PUFA, but because the PUFA were dominated by 18:2 (n-6) there was a relatively low n-3/n-6 ratio (0.64). The principal SFA included 16:0 and 18:0 (Table 4).

The oats/EPA/AA feed was enriched in AA and EPA and contained less DHA (Table 4). The major MUFA were 18:1(n-9)c and 16:1 (n-7)c. The EPA/AA ratio was 1.3, the ratio (n-3)/(n-6) 1.1, and the ratio DHA/EPA 0.64. Palmitic acid, 16:0, was the major SFA; 14:0 and 18:0 were relatively lower. The DHA Selco feed was characterized by greater DHA with relatively less AA and EPA (Table 4). The DHA/EPA ratio was 3.9, and the EPA/AA ratio 3.2. This diet was characterized by low (n-6) fatty acids and the sum of the (n-6) fatty acids was less than the sum of the (n-3) fatty acids. The MUFA included primarily 18:1(n-9)c, 16:1 (n-7)c, and 18:1 (n-7)c. Palmitic acid (16:0) was the major SFA; 14:0 and 18:0 were lower.

Artemia fatty acid composition

Artemia fed the algal diet had low AA, EPA and DHA (Table 4, Fig. 2). The principal fatty acids in *Artemia* fed algae included 18:1(n-9)c, 18:1(n-7)c, 18:2(n-6), 16:0, and 18:0. MUFA were a greater percentage than total PUFA. The sum (n-3) was less than the sum (n-6). The EPA/AA ratio was 1.9, the DHA/EPA ratio only 0.04.

Artemia fed the oats/EPA/AA diet had high AA and EPA and less DHA (Table 4, Fig. 2). This pattern was reflected in the sum PUFA, an EPA/AA ratio of 1.3, and a DHA/EPA ratio of 0.47. Levels of 18:1(n-9)c were greater than those of 18:1 (n-7)c, and 16:1 (n-7)c. The major SFA included 16:0, 18:0, and 14.0.

Artemia fed the DHA Selco diet had low AA and high DHA relative to those fed the other enrichment diet (Table 4, Fig. 2). EPA was 8.7% of total FA. The low AA was reflected in a higher EPA/AA ratio of 3.2, and the high DHA in a high DHA/EPA ratio (1.8). The sum PUFA was similar to the sum PUFA for the *Artemia* fed the oat/EPA/AA diet, but the sum (n-3) was higher. MUFA compositions were similar in the two enrichment diets; the sum MUFA was almost identical. The sum SFA was also identical for the two enrichment diets, as was the SFA composition.

Table 3. Percentage lipid class composition of feed (for feeding to *Artemia*) and *Artemia* fed for 5 days.

	n	Wax esters	Triacyglycerols	Free fatty acids	Sterols	Polar lipids	Lipid as mg g^{-1} dry mass
Feeds only							
Tetraselmis suecica	9	3.1 ± 1.9	2.3 ± 1.8	1.5 ± 1.8	1.7 ± 1.2	91.4 ± 4.4	108.8 ± 22.0
Oat/EPA/AA	2	–	87.8 ± 1.4	0.0 ± 0.0	1.1 ± 0.2	11.2 ± 1.6	287.8 ± 6.7
DHA-selco	2	0.2 ± 0.0	85.3 ± 0.2	0.5 ± 0.0	0.5 ± 0.0	13.6 ± 0.1	806.1 ± 38.8
***Artemia*, fed as follows:**							
Tetraselmis suecica	2	0.3 ± 0.2	4.3 ± 0.4	0.2 ± 0 1	4 6 ± 1.0	90.6 ± 0.8	97.9 ± 4.0
Oats/EPA/AA	3	0.1 ± 0 1	70.6 ± 1 9	1.8 ± 0.7	2.2 ± 0.4	25.3 ± 1.8	258.9 ± 5.8
DHA-selco	3	–	71.8 ± 3.3	4.4 ± 1.5	1.6 ± 0.5	22.3 ± 1.3	296.5 ± 31.7

presented as mean ± sd; first 4 days *Artemia* fed oat bran/wheat germ/lecithin (50:6:4) only; next 24 hrs *Artemia* fed diets as specified; –, below detection.

Discussion

Lipid classes and fatty acids—southern rock lobster (preservation experiment)

Formalin-preserved samples of newly hatched phyllosomata differed significantly in fatty acid composition from samples stored otherwise (in particular, they had lower AA, EPA, and DHA, Table 2). PUFA profiles of these formalin-treated samples were seriously compromised, so formalin should not be used for samples to be analyzed for lipid and fatty acid composition. In contrast, the ethanol-preserved newly hatched phyllosoma samples did not differ in fatty acid profile from fresh extracted, frozen, or lyophilized ones (Table 2). We were therefore able to use ethanol-stored phyllosomata (from Tasmanian waters) in our study. Because ethanol extracts lipid, the sample plus all ethanol must be used in the Bligh-Dyer extraction. The extraction is therefore performed with chloroform, ethanol, and water, where ethanol replaces methanol. Not only were the fatty acid profiles of ethanol-preserved samples nearly identical to those of fresh, frozen, and lyophilized samples (Table 2), the level of FFA in the lipid class profile was also very low (Table 1). High FFA levels would indicate lipid degradation. In the case of historical ethanol-preserved samples, in which the ethanol preservative may have been exchanged, some of the lipid may be missing, so care is needed in selecting preserved samples for lipid analysis.

Lipid classes—wild southern rock lobster

PL was the major lipid class in the southern rock lobster (adult, newly hatched phyllosomata, phyllosomata from New Zealand and Tasmania, and pueruli from Tasmania) and comprised 82.5–95.9% (of total lipid) in all samples. Our lipid class data for the newly hatched phyllosomata are in general agreement with those of Smith (1999) in that PL is the major lipid class. Smith (1999) noted low levels of hydrocarbon (3–6%), no DG, low TAG (2.1–2.7%), and somewhat higher sterol (8.7–10.9%) in newly hatched phyllosomata of *J. edwardsii*. Some of these differences may be due to small variations in methods. Pearce (1997) found PL to be the major lipid class in pueruli of *J. edwardsii*, which also had low levels of DG and TAG. TAG increased in relative percent in the settled pueruli, perhaps in preparation for the moult (Pearce, 1997). DG was the second most abundant lipid class in most of these larval rock lobsters (stage V–XI and nektonic pueruli); ST (>95% cholesterol) was usually third in abundance.

PL (mostly phospholipid) and ST primarily function as membrane structural lipids (Pond & Sargent, 1998), but in certain organisms, such as the Antarctic krill *Euphausia superba*, PL are depleted as energy reserves, in addition to depletion of TAG (Virtue *et al.*, 1993; Hagen *et al.*, 1996). Interestingly, PL is also depleted in *J. edwardsii* pueruli (Pearce, 1997; Jeffs *et al.*, 2001a), in keeping with the role of lipid as the primary energy store of nektonic pueruli of *J. edwardsii* (Jeffs *et al.*, 1999). DG also may play a less significant secondary role as energy reserve in *J. edwardsii* pueruli (Jeffs *et al.*, 2001a). Both PL and DG may play a similar role in later-stage phyllosomata (Table 1), because almost no TAG or WE

Table 4. Percentage fatty acid composition of feed (for feeding to *Artemia*) and *Artemia* fed for 5 days.

	Feeds only			*Artemia*, fed as follows:		
	Tetraselmis suecica	Oats/EPA/AA	DHA-selco	*Tetraselmis suecica*	Oats/EPA/AA	DHA-selco
14:0	0.5 ± 0.1	5.6 ± 0.2	3.3 ± 0.0	0.4 ± 0.1	2.2 ± 0.0	1.6 ± 0.1
15:0	–	0.4 ± 0.1	1.0 ± 0.0	–	0.1 ± 0.0	0.3 ± 0.1
C_{16} PUFA	3.0 ± 6.0	1.3 ± 0.2	0.1 ± 0.1	1.3 ± 0.9	0.5 ± 0.1	0.2 ± 0.1
16:1(n-9)c	1.3 ± 0.3	0.1 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.6 ± 0.0
16:1(n-7)c	2.0 ± 0.6	6.6 ± 0.6	4.2 ± 0.2	1.5 ± 0.1	4.8 ± 0.1	4.1 ± 0.1
16:0	21.3 ± 4.1	17.1 ± 0.4	21.4 ± 0.6	13.9 ± 0.3	10.3 ± 0.1	11.0 ± 0.2
a17:0/17:1	0.3 ± 0.3	0.1 ± 0.1	0.7 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.8 ± 0.1
17:0	0.2 ± 0.3	0.5 ± 0.0	1.3 ± 0.0	0.7 ± 0.0	0.4 ± 0.0	0.6 ± 0.0
18:3(n-6)	1.8 ± 0.5	–	–	1.8 ± 0.1	0.7 ± 0.0	0.2 ± 0.0
18:4(n-3)	9.0 ± 1.9	1.8 ± 0.1	0.9 ± 0.2	4.5 ± 0.3	2.1 ± 0.1	1.6 ± 0.1
18:2(n-6)	19.2 ± 5.2	10.0 ± 0.3	5.4 ± 0.2	17.1 ± 0.5	16.7 ± 0.2	13.2 ± 0.1
18:1(n-9)c/18:3(n-3)	22.9 ± 4.5	14.2 ± 0.2	18.0 ± 3.0	23.7 ± 0.1	22.2 ± 0.3	24.0 ± 0.1
18:1(n-7)c	4.1 ± 2.2	2.8 ± 0.0	3.0 ± 0.2	10.3 ± 0.5	4.1 ± 0.1	4.7 ± 0.2
18:0	2.1 ± 2.4	4.4 ± 0.1	6.0 ± 0.2	9.4 ± 0.3	4.1 ± 0.0	4.2 ± 0.1
20:4(n-6) - AA	1.5 ± 0.4	8.9 ± 0.0	1.7 ± 0.1	3.4 ± 0.8	8.9 ± 0.2	2.7 ± 0.7
20:5(n-3) - EPA	5.1 ± 0.7	11.7 ± 0.3	5.3 ± 0.3	6.3 ± 0.2	11.3 ± 0.1	8.7 ± 0.3
20:1(n-11)c	1.3 ± 0.5	0.8 ± 0.0	1.0 ± 0.0	0.9 ± 0.0	0.6 ± 0.0	0.7 ± 0.0
22:5(n-6)	–	0.3 ± 0.1	1.6 ± 0.1	0.1 ± 0.1	0.2 ± 0.0	1.1 ± 0.1
22:6(n-3) - DHA	0.2 ± 0.2	7.5 ± 0.0	20.7 ± 0.6	0.0 ± 0.1	5.3 ± 0.3	15.4 ± 1.1
22:5(n-3) - DPA	0.1 ± 0.2	1.5 ± 0.0	0.9 ± 0.1	–	1.0 ± 0.1	0.9 ± 0.0
22:1	1.8 ± 4.8	0.5 ± 0.0	0.3 ± 0.0	–	0.3 ± 0.0	0.2 ± 0.0
Other	2.3	4.0	3.0	3.9	3.4	3.0
Sum SFA	25.6 ± 2.5	28.9 ± 0.9	34.6 ± 1.0	26.2 ± 0.6	18.7 ± 0.1	19.9 ± 0.1
Sum MUFA	33.6 ± 6.5	25.8 ± 0.4	27.9 ± 2.5	37.5 ± 0.3	33.2 ± 0.5	34.9 ± 0.2
Sum PUFA	40.7 ± 6.2	44.9 ± 1.1	37.4 ± 1.5	36.1 ± 0.6	48.1 ± 0.4	45.1 ± 0.3
Sum (n-3)	14.7 ± 2.0	23.0 ± 0.4	28.1 ± 1.1	11.3 ± 0.5	20.3 ± 0.5	27.0 ± 0.8
Sum (n-6)	23.0 ± 5.4	20.5 ± 0.5	9.2 ± 0.4	23.5 ± 0.3	27.2 ± 0.2	17.8 ± 0.7
Ratio (n-3)/(n-6)	0.64	1.12	3.05	0.48	0.75	1.51
Ratio EPA/AA	3.32	1.32	3.17	1.85	1.27	3.19
Ratio DHA/EPA	0.04	0.64	3.93	0.04	0.47	1.77

presented as mean ± sd; details provided in Table 3; first 4 days *Artemia* fed oat bran/wheat germ/lecithin (50:6:4) only; next 24 hrs *Artemia* fed diets as specified; –, below detection; tr, trace (<0.05%); AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; Other includes components present at <1%: i15:0, a15:0, 16:1(n-7)t, i17:0, 18:1(n-7)t, 20:4(n-3), 20:3(n-6), 20:2(n-6), 20:1(n-7)c, 20:0, 22:4(n-6), 22:0, 24:1, 24:0.

were detected in them. TAG, a short-term energy-reserve molecule, is generally the most common lipid reserve in marine organisms, whereas WE a long-term energy-reserve molecule, is less common (Benson & Lee, 1975). The occurrence of PL as the major lipid class in *J. edwardsii* phyllosomata and nektonic pueruli could be due to its transparency *in vivo*, which could provide protection to the phyllosomata from predation in the water column, as proposed for puerulus by Jeffs *et al.* (2001a).

Cholesterol is necessary for the normal growth and survival of juvenile American lobsters, *Homarus americanus* (D'Abramo *et al.*, 1984). Cholesterol biosynthesis does not occur from [^{14}C]-labeled precursors in spiny lobsters (Teshima & Kanazawa, 1971). Replacement of cholesterol with phytosterols (mostly β-sitosterol) did not result in satisfactory growth and survival of *H. americanus* (D'Abramo *et al.*, 1984). In our study (Table 1), ST (>95% cholesterol) levels are higher in adults and newly hatched and early stage phyllosomata (6.2–12.0%) than in later stages (stages V–XI) (0.8–5.5%) and nektonic pueruli (4.1%). The reason(s) for this difference remain(s) to be determined.

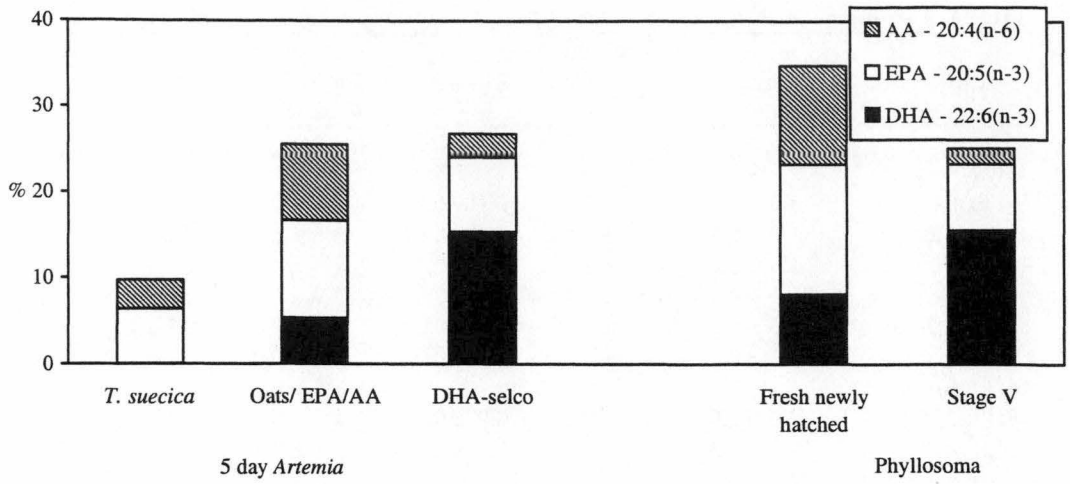

Fig. 2. Percent composition of arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in enriched *Artemia* and southern rock lobster, *Jasus edwardsii*, phyllosomata.

Although lipid is relatively low in adults and newly hatched phyllosomata (31.0–56.5 mg g^{-1} lipid, as dry mass), it is substantially greater in later-stage phyllosomata (94.6–347.3 mg g^{-1} lipid) (Table 1). Lipid levels increased in later-stage phyllosomata (New Zealand) from 185.7–242.1 mg g^{-1} (stage V–VIII) to 279.4–344.0 mg g^{-1} in stage IX–XI. Total lipid in nektonic pueruli (34.8 mg g^{-1}) was low in comparison. This large decrease in lipid is in keeping with evidence that pueruli do not feed (Kittaka, 1990). Fat bodies decrease in size as lobsters develop from the transparent to the pigmented puerulus stage (Takahashi *et al.*, 1994). According to Lemmens (1994), in *Panulirus cygnus*, energy reserves are accumulated during the last phyllosoma stage and are used during the non-feeding puerulus stage.

Fatty acids—wild southern rock lobster

One of the most obvious characteristics of the fatty acid profile of newly hatched phyllosomata is their high AA levels (11.6–11.9% of total FA, not including the formalin-preserved specimens) relative to later-stage phyllosomata (1.4–3.1%) (Table 2). Smith (1999) first noted elevated levels of AA in newly hatched stage I phyllosomata of *J. edwardsii* (6.8–9.3% of total FA). It was pointed out that AA is an essential fatty acid usually only found in small quantities in marine organisms (Olsen, 1998). Smith (1999) suggested that the maternal diet source before spawning influences the fatty acid composition of the total lipid fraction of newly hatched phyllosomata. Adult *J. edwardsii* contain 7.3% AA (Table 2) (Nichols *et al.*, 1998c). The similarity of the fatty acid profiles of adult and newly hatched *J. edwardsii* phyllosomata confirms this suggestion. AA may also play a specific role in crustacean nutrition, because consistent levels are usually present at the earliest stages of larval development (Chapelle, 1986).

Newly hatched and later-stage phyllosomata also differ in other notable ways, namely the higher EPA and lower DHA at the later stages (Table 2). These fatty acids are both considered essential because they are not synthesized by the animal from acetate-[^{14}C], as has been demonstrated in European lobster (Zandee, 1967). Both [^{14}C] EPA and [^{14}C] DHA were incorporated at high levels in adult *Panulirus japonicus* into phospholipids such as phosphatidylcholine and phosphatidylinositol (Kanazawa & Koshio, 1994). EPA and DHA must be synthesized from fatty acid precursors or be obtained from the diet. In the case of newly hatched phyllosomata, they are derived from the adult broodstock. Marine oils (cod liver oil) that contain these essential PUFA have been demonstrated to be superior to vegetable oils (corn oil or hydrogenated coconut oil, devoid of EPA and DHA) in the diet of *Homarus americanus* (Castell & Covey, 1976).

EPA is typically found in higher proportion in diatoms, whereas dinoflagellates and other flagellates contain more DHA than EPA. Diatoms are characterized by high EPA and high 16:1/16:0 ratio as noted in a recent food-chain study (Nelson *et al.*, 2000). Although both Tasmanian and New Zealand phyllosomata show a higher EPA-to-AA ratio than do newly hatched phyllosomata, the 16:1/16:0 ratio is low. The higher DHA-to-EPA ratios in the later-stage phyllosomata than in early stages (Table 2) probably derive from feeding in the pelagic plankton environment and may reflect input from dinoflagellates or other plankton rich in DHA.

The C_{18} omega-3 PUFA 18:4(n-3) was only detected in the later-stage phyllosomata (Table 2). This PUFA is a major fatty acid in the prymnesiophyte *Isochrysis* sp. (T-ISO) and in the cryptomonad *Chroomonas salina* (Volkman *et al.*, 1989). It may be passing though the food chain to later-stage phyllosomata.

High levels of OA in later-stage phyllosomata indicate a zooplankton-rich diet. Herbivorous salps (*Salpa thompsoni*, Urochordata), for example, have only 3–7% oleic acid (Phleger *et al.*, 2000), as a result of their phytoplankton-rich diet. The transparent phyllosomata may feed on transparent gelatinous organisms in the open ocean (D. Ritz and T Koslow, personal communication). Signature lipid profiles such as those of OA (Table 2) for later-stage *J. edwardsii* phyllosomata are more closely correlated with those of cnidarian jellies than with those of salps (Nelson *et al.*, 2000); unpublished data). For example, high OA (18.6–26.4%) levels are characteristic of certain cnidarian jellies, such as *Calycopsis borchgrevinki* and *Stygiomedusa gigantea* (Nelson *et al.*, 2000), but the EPA-to-DHA ratios do not match. More signature lipid profiles are needed for gelatinous zooplankton collected from waters near where the phyllosomata are located. In addition, our data provide evidence (<1% branched-chain, odd-numbered fatty acids; Table 2 footnote) that bacteria are not a significant component in the phyllosoma diet.

Our fatty acid data for AA, EPA, and DHA of *J. edwardsii* puerulus (Table 2) are in agreement with those of Pearce (1997). Marked increases in AA, EPA, and DHA were reported in the early-instar juveniles (Pearce, 1997), and these increases were attributed to diet. PUFA deficiency can be an important problem in aquaculture operations, resulting in early mortality and various ailments.

Feeding experiment—Artemia enrichments

The oats/EPA/AA diet more successfully enriched *Artemia*, after 24 h, in AA (from 3.4% to 8.9% of total FA) and EPA (from 6.3% to 11.3%) than did the *Tetraselmis* diet (Table 4). It increased TAG levels to 71% of total lipid; the algal diet yielded only 4.3% (Table 3). This diet program has excellent potential for providing EPA- and AA-enriched *Artemia* to newly hatched phyllosomata, which contain elevated levels of these two PUFA (11% AA, 15% EPA; Table 2, Fig. 2).

During later-stage phyllosoma development (stages IV to XI, Table 2), a shift in the ratios of the essential PUFA occurs; both the EPA-to-AA and the DHA-to-EPA ratios increase, suggesting a decrease in the requirement for AA while DHA assumes greater importance.

A number of studies have demonstrated enrichment of *Artemia* with essential omega-3 fatty acids (in particular DHA), primarily to improve fish larval culture. Newly hatched *Artemia* nauplii took up omega-3 highly unsaturated fatty acids (HUFA) homogenized with raw egg yolk, water, and bakers yeast and improved the growth of fish (Watanabe *et al.*, 1982) that fed on them. Increased survival and biomass of the larval sea bass *Dicentrarchus labrax* were attributed to the omega-3 HUFA content of enriched *Artemia* nauplii in their diet (Van Ballaer *et al.*, 1985). *Artemia* enrichment was reviewed by Léger *et al.* (1987a), and the importance of DHA enrichment was stressed, because DHA is usually absent from *Artemia* nauplii. Phospholipid was reported to be the most efficient lipid class for delivery of DHA to *Artemia* nauplii (Harel *et al.*, 1999). Feeding experiments with *J. edwardsii* phyllosoma larvae have used *Artemia* as feed for the first two stages and then mussels, *Mytilus edulis*, for the later stages (Kittaka, 1994a). *Artemia* (2–3 mm) reared on microalgae

were found to be suitable food for stage I to VI *J. edwardsii* phyllosoma larvae (Tong *et al.*, 1997).

The DHA Selco diet successfully enriched *Artemia* after 24 h to 15.4% DHA (of total FA), compared to 0.2% for the algal diet and 5.2% for the oats/EPA/AA diet (Table 4, Fig. 2). The DHA-to-EPA ratios were increased to 2.2 in *Artemia* fed DHA Selco from 0.03 and 0.52 in *Artemia* fed oats/EPA/AA (Table 4).

The lipid and fatty acid data presented in this report confirm the possibility of designing relatively simple and low-cost *Artemia* diets with PUFA profiles that may offer potential for use with rock-lobster larvae. The PUFA profiles of later-stage phyllosomata of rock lobster, obtained here for the first time, change markedly during development. High levels of the essential PUFA were obtained with *Artemia* fed simple diet mixes based on off-the-shelf products. Also, for the first time *Artemia* were simultaneously enriched with all three essential PUFA, EPA, DHA, and in particular AA. These enrichment results for DHA, EPA, and AA in *Artemia* should be of potential use for aquaculture, more specifically larviculture, of *J. edwardsii*, particularly in the earlier stages. The use of off-the-shelf products for enrichment of *Artemia* may be better suited to later-stage phyllosomata. Feeding trials of phyllosomata and early juveniles using *Artemia* fed or enriched with these or similar diets are needed to ascertain the suitability of the diets and to confirm whether the survival and growth rates of rock-lobster larvae can be improved.

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Appendix Two

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Marked depletion of polar lipid and non-essential fatty acids following settlement by post-larvae of the spiny lobster *Jasus verreauxi*

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Abstract

The development from the non-feeding post-larva (puerulus) to the first instar juvenile of spiny lobsters is highly demanding energetically. These demands may greatly compromise the energy reserves of the lobsters following settlement, leading to reduced growth and survival in the wild and also in aquaculture. Therefore, the lipid class and fatty acid composition of wild caught pueruli and first instar juveniles of the spiny lobster *Jasus verreauxi* (H. Milne Edwards, 1851) were analyzed by thin layer chromatography–flame ionization detection and capillary gas chromatography. Pueruli contained substantially more lipid than first instar juveniles (mean difference = 3.5 mg, or 41.9%) and most of this difference was due to the presence of greater amounts of polar lipid (mean difference = 3.9 mg or 49.2%) in pueruli. First instar juveniles contained significantly more triacylglycerol (mean = 0.2 mg), consistent with the polar lipid being converted to a more readily metabolized lipid class in the hepatopancreas. These results indicate that polar lipid is the major energy store during the non-feeding puerulus stage of spiny lobsters from the genus *Jasus*. Overall, the essential polyunsaturated fatty acids, linoleic, docosahexaenoic and eicosapentaenoic acids did not show a significant decrease between the two developmental stages despite the absence of feeding. However, significant reductions in the abundance of both saturated and monounsaturated fatty acids between the two stages were identified (decrease of 811 and 783 μg per individual, respectively). This suggested that selective depletion of non-essential fatty acids may be occurring, with resultant sparing of the essential fatty acids. Supplying diets rich in these depleted fatty acids and in particular the essential fatty acids, preferably in polar lipid, is likely to result in increased survival and growth of *J. verreauxi* and other spiny lobsters from first instar juveniles in aquaculture.

Keywords: Post larvae; Pueruli; Spiny lobster; Lipid; *Jasus verreauxi*; Polar lipid; Fatty acids

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1. Introduction

The non-feeding post-larvae, or pueruli, of spiny lobsters appears to swim over considerable distances, up to 100s of km, from the open ocean into shallow coastal waters where they settle (Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a; Jeffs *et al.*, 2001b). Polar lipid (PL) are the primary energy storage component of the puerulus of the spiny lobster *Jasus edwardsii*, and diacylglycerol (DG) appear to play a much less important secondary role (Pearce, 1997; Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a; Jeffs *et al.*, 2001b; Phleger *et al.*, 2001). There are indications that a substantial proportion of settling pueruli may be too exhausted to undergo the post-settlement development and subsequent moult to become first instar juveniles (Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a; Jeffs *et al.*, 2001b). Following the moult, the fully developed mouthparts of the first instar juvenile enable the lobster to commence benthic feeding

(Nishida *et al.*, 1990). Very little is known about the energy requirements of this development phase which may last up to 4 weeks after settlement (Booth & Kittaka, 2000; Kittaka, 2000). Histological studies of *J. edwardsii* indicate that large quantities of stored PL are mobilized by the hepatopancreas to fuel the substantial morphological changes during this period (Nishida *et al.*, 1995; Jeffs *et al.*, 2001a). This was confirmed by some preliminary lipid analysis of developing pueruli of *J. edwardsii* (Pearce, 1997). Therefore, it is very likely that following moulting the first instar juvenile has a severe energy deficit that can only be solved by immediate benthic feeding. In the aquaculture of spiny lobsters this is a critical phase when most mortality of cultured pueruli occurs, probably as a direct result of this energy deficit (Kittaka, 2000; Jeffs, 2001). Supplying a formulated feed that contains an appropriate energy form at this critical time may help to overcome this difficulty for this emerging aquaculture industry. The aim of this study was to identify the energy use of the pueruli from settlement to first instar juvenile in *J. verreauxi*, a spiny lobster species of intense aquaculture interest in temperate parts of the Southern Hemisphere (Booth & Kittaka, 2000; Kittaka, 2000). This work also represents the first published study on the lipid composition of this species of lobster.

2. Materials and methods

2.1. Animal collection

Eight puerulus and 15 first instar juveniles of *J. verreauxi* were collected on 15 October 1999 off the east coast of New South Wales, near Sydney, Australia, using artificial seaweed collectors (Montgomery & Craig, 1998). Three replicate collectors floating on the surface of the sea were moored in 10–12 m of water, at each of three sites, spaced over a distance of approximately 18 km. The pueruli were all clear with no visible sign of development of the digestive tract indicating they had very recently settled from the plankton, probably within the previous 48 hours. The first instar juveniles all had darkly pigmented carapaces and could have settled out on the collectors up to 29 days prior to collection, i.e. 16 September 1999 when the collectors were last cleared. All animals were frozen live (–20°C) shortly after collection and transported to the laboratory for analyses.

2.2. Sample analyses

Each animal was weighed frozen and then lyophilized to a constant mass.

The lipid from individual animals was quantitatively extracted using a modified Bligh and Dyer (1959) one-phase methanol/chloroform/water extraction. The lipid extract was then concentrated using rotary evaporation and blown down to a constant mass with nitrogen gas. An aliquot of the total solvent extract was then analyzed using an Iatroscan MK V TH10 thin layer chromatography-flame ionization detector (TLC-FID) analyzer (Tokyo, Japan) to determine the abundance of individual lipid classes (Volkman & Nichols, 1991). Samples were applied in duplicate or triplicate to silica gel SIII Chromarods (5 μm particle size) using 1 μl disposable micropipettes. Chromarods were developed using the solvent system hexane/diethyl ether/acetic acid (60:17:0.2, by vol.) a mobile phase that resolves non-polar compounds such as wax ester (WE), triacylglycerol (TAG), free fatty acid (FFA) and sterol (ST). A second non-polar solvent system of hexane/diethyl ether (96:4 v/v) was also used for selected samples to separate hydrocarbon from WE, and TAG from diacylglyceryl ether (DAGE). After development, the chromarods were oven-dried and analyzed immediately. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, WE (derived from fish oil), TAG (derived from fish oil) and DAGE (purified from shark liver oil); 0.1–10 μg range]. Peaks were quantified on a pc using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ±10% or better (Volkman & Nichols, 1991).

An aliquot of each lipid extract was treated with methanol/hydrochloric acid/chloroform (10:1:1, by vol; 80°C, 2 h). The fatty acid methyl esters (FAME) products were extracted

into hexane/chloroform (4:1, v/v, 3 × 1.5 ml) and then treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 50 μl, 60°C, 1 h) to convert sterols to their corresponding trimethylsilyl ethers. Gas chromatographic (GC) analyses of methyl esters of FAME and sterol were performed with a Hewlett Packard 5890A GC (Avondale, PA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of methyl nonadecanoate and methyl tricosanoate internal standards, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1} and finally to 300°C at 5°C min^{-1}. Peaks were quantified with Waters Millennium software (Milford, MA, USA). Individual components were identified using mass spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. Gas chromatographic results are subject to an error of ±5% of individual component abundance. Gas chromatographic-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

2.3. Statistical analyses

Statistical comparisons among the mean values for the quantities of lipid classes and fatty acids (FA) from individual pueruli and first instar juveniles were conducted with a Student's *t*-test when sample variances were found to be homogeneous with the F_{max} test (Sokal & Rohlf, 1995). Otherwise the comparison of means was conducted with a Welch's approximate *t*-test. Means were judged to be significantly different where $\alpha = 0.05$. All quantitative data presented are mean values for individual animals.

3. Results

3.1. Lipid classes

The mean water content, and the wet and dry mass of pueruli were significantly less than for first instar juveniles (Table 1). The increased mass of the first instar juveniles in the absence of feeding was due to calcium accumulation which is used for exoskeleton hardening (Lemmens, 1995), consequently most of the following lipid results have been reported as absolute mass rather than a percentage of dry body mass. Pueruli contained significantly greater quantities of lipid, 3.5 mg, or 41.9 % more lipid than first instar juveniles (Table 1). PL on average was the major lipid class present in both the pueruli and first instar juveniles, accounting for 93.8% and 82.1% of total lipids for each group of animals respectively. Pueruli contained significantly more PL on average than first instar juveniles, 3.9 mg, or 49.2% more PL than first instar juveniles (Table 1). ST was the second major lipid class, but accounted for only 5.3% of total lipid in pueruli and 7.5% in first instar juveniles and this was not significantly different. TAG were absent in pueruli and significantly greater quantities were found in the first instar juveniles (0.2 mg) (Table 1). FFA and DAGE were present only in very small amounts, if at all. DG were absent from both developmental stages.

3.2. Fatty acids (FA)

For most fatty acids (FA) there was a general trend of decreasing abundance from pueruli to first instar juveniles corresponding with the overall decrease in lipid mass between the two developmental stages (Fig. 1). The average amount of total saturated (SFA) and monounsaturated FA (MUFA) significantly decreased between the two stages (decrease of 811 and 783 μg, respectively), but not the polyunsaturated FA (PUFA). The largest significant decreases occurred in the following: SFA, 16:0 (463 μg), 18:0 (227 μg), 14:0 (75 μg); MUFA, 18:1(n-9)c (355 μg), 16:1(n-7)c (158 μg), 20:1(n-11/9)c (59 μg); and PUFA 18:4(n-3) (31 μg), 18:3(n-6) (17 μg). The essential PUFA, docosahexaenoic acid [DHA, 22:6(n-3)], eicosapentaenoic acid [EPA, 20:5(n-3)], arachidonic acid [AA, 20:4(n-6)] and linoleic

Table 1
Comparison of the lipid content of recently settled pueruli and first instar juveniles of *Jasus verreauxi*.

	Total wet body (g)	Total dry body (g)	Water (g)	Total lipid (mg)
Pueruli (n= 8)	0.37 ± 0.02	0.07 ± 0.00	0.30 ± 0.02	8.34 ± 1.46
First instar juveniles (n = 15)	0.59 ± 0.06	0.12 ± 0.02	0.47 ± 0.05	4.84 ± 0.59
t value	t_{18} = 3.6	t_{16} = 3.2	t_{19} = 3.6	t_{10} = 2.4
P value	< 0.005	< 0.005	< 0.005	< 0.05
Mean Difference	0.22	0.05	0.17	-3.50
	Triacylglycerol (mg)	Free fatty acid (mg)	Sterol (mg)	Polar lipid (mg)
Pueruli (n= 8)	0.00 ± 0.00	0.08 ± 0.02	0.44 ± 0.06	7.82 ± 1.38
First instar juveniles (n = 15)	0.22 ± 0.08	0.28 ± 0.13	0.36 ± 0.03	3.97 ± 0.45
t value	t_{14} = 2.8	t_{14} = 1.7	t_{10} = 1.2	t_9 = 2.8
P value	< 0.025	> 0.05	> 0.05	< 0.025
Mean Difference	0.22	Nil	Nil	-3.85

All values are mean total mass (g or mg) derived from sampling individual animals ± standard error.

acid [LNA, 18:2(n-6)] unlike the SFA and the MUFA were not significantly different in first instar juvenile stages when compared to pueruli.

4. Discussion

The predominance of PL as the major lipid class in *J. verreauxi* pueruli (93.8% of total lipid) (Table 1) is essentially the same as that reported for *J. edwardsii* pueruli (86–96%) (Jeffs *et al.*, 2001a). Only the PL fraction decreased (by 49.2%) between the settling puerulus and the first instar juvenile stages, suggesting that PL is the primary energy store for the non-feeding pueruli stage of *J. verreauxi*. PL has also been found to be the primary energy store of *J. edwardsii* pueruli and DG appears to play a much less important role during the period of swimming onshore prior to settlement (Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a; Jeffs *et al.*, 2001b). However, DG were neither detected in the puerulus or first instar juvenile of *J. verreauxi*. TAG were absent in the recently settled pueruli of *J. verreauxi*, but although only a minor component (4.5% of total lipid), was found in significantly greater quantities (on average 0.2 mg) in the first instar juveniles. This may be related to the conversion of stored PL to TAG by the hepatopancreas, in order to fuel the substantial morphological changes occurring at this time. This transition of lipid stores has also been observed by histological and biochemical studies in *J. edwardsii* pueruli (Nishida *et al.*, 1995; Pearce, 1997; Jeffs *et al.*, 2001a). Overall, these results suggest that PL plays a major energy storage role during the non-feeding puerulus stage of spiny lobsters of the genus *Jasus*. The presence of disproportionately large quantities of PL in the adults and larvae of a number spiny lobster species, strongly suggests that PL may play a dominant role in energy storage throughout the life cycle of spiny lobsters (Nichols *et al.*, 1998c; Phleger *et al.*, 2001). This would also explain why previous attempts to use TAG as a measure of spiny lobster condition have consistently failed (Robertson *et al.*, 2000). The use of PL for energy storage would be unusual, as most other crustaceans commonly use TAG, ST and WE for energy storage (Sargent, 1976; Anger, 1998). In the Antarctic krill *Euphausia superba*, PL are depleted as energy reserves in addition to depletion of TAG (Virtue *et al.*, 1993; Hagen *et al.*, 1996).

The marked decrease in PL between the two developmental stages of *J. verreauxi* were associated with significant decreases in total SFA and MUFA, and in particular the FA, 16:0, 18:0, 18:1(n-9)c and 16:1(n-7)c. Overall, PUFA and the essential FA (Kanazawa, 2000), EPA, DHA, AA and LNA did not significantly decrease between the two stages. This suggested that selective use of non-essential FA may be occurring, with resultant sparing of the essential PUFA. Selective depletion of FA during starvation stress has been observed in post-spawning Pacific pink salmon (Phleger *et al.*, 1995), and appears to be a common phenomenon among vertebrates (Raclot & Groscolas, 1993). Furthermore, comparison of the FA profile of *J. verreauxi* pueruli and first instar juveniles with those reported

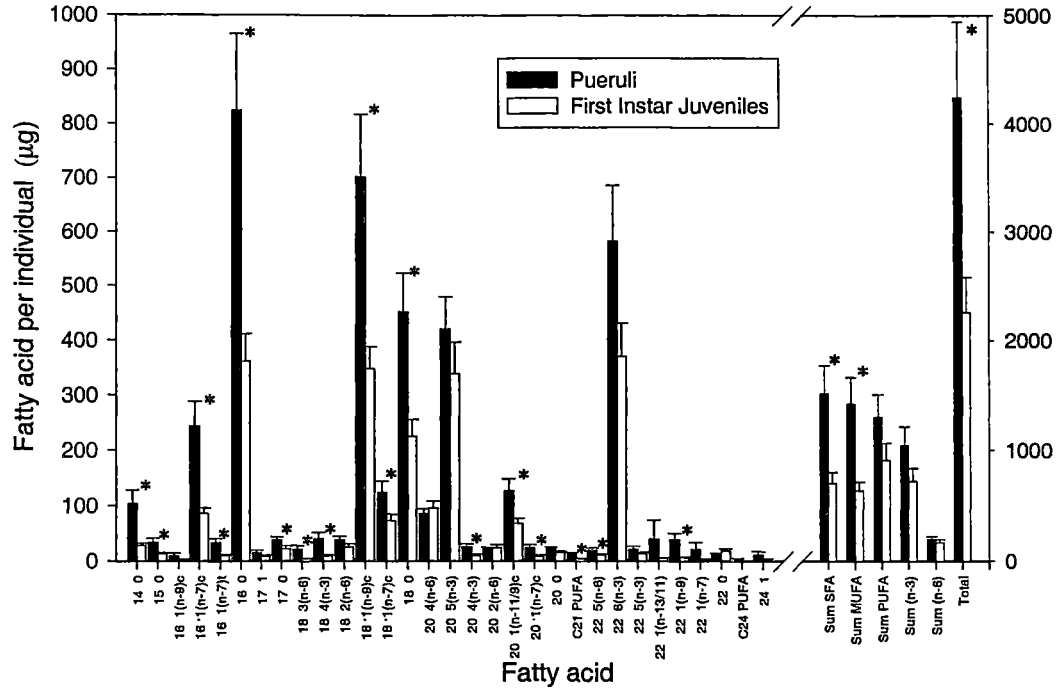

Fig. 1. Fatty acid profile of pueruli and first instar juveniles of *Jasus verreauxi*. Data are presented as mean µg of fatty acid +/- standard error per individual. Significantly different means are indicated by *.

for pueruli and adults of the closely related *J. edwardsii* suggest that essential PUFA are much reduced in these earlier developmental stages compared to the adult (Nichols *et al.*, 1998c; Phleger *et al.*, 2001). Unfortunately, no data on the FA profile of adult *J. verreauxi* are available for direct comparison, however, the FA profiles of spiny lobsters, including those cited for *J. edwardsii* of different species are similar (Nichols *et al.*, 1998c). Total PUFA represents 46.5% of the FA of adult *J. edwardsii*, and only 31.2% in the pueruli and 39.8% in the first instar juveniles of *J. verreauxi*. This is largely because the essential FA, AA, LNA, EPA and DHA, are all substantially lower in pueruli and first instar juveniles. Future studies on the biochemistry of PL as an energy reserve in lobsters should identify the specific PL classes and the FA composition of each PL class in both the pueruli and first instar juveniles. PL from the krill *E. superba*, for example, includes both phosphatidylcholine and phosphatidylethanolamine with higher proportions of EPA (Mayzaud, 1997).

These results suggest that PL are the major energy store, and that their essential PUFA, including LNA, EPA, DHA and AA are all depleted in first instar juvenile spiny lobsters following an extended period of non-feeding associated with energetically demanding swimming activity and substantial morphological changes. This energy deficit may be responsible for the high mortalities of first instar juveniles that are commonly experienced in aquaculture and also probably in the wild (Kittaka, 2000; Jeffs *et al.*, 2001a; Jeffs *et al.*, 2001b). Supplying diets to first instar juveniles that are rich in these essential PUFA, preferably in PL, is likely to result in increased survival and growth of *J. verreauxi* and other spiny lobsters in aquaculture.

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Appendix Three

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Changes in gut content and composition of juvenile *Artemia* after oil enrichment and during starvation

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Abstract

Some larval crustacean tear *Artemia* to pieces before ingestion, this results in the loss of gut content, which may partly negate the benefits of enrichment with essential fatty acids (EFA). Therefore, the influence of gut content on the lipid composition of juvenile *Artemia* (5 days old) was examined by starvation alone or starvation with forced gut evacuation using 20–30-μm plastic beads. *Artemia* gut content, at 3 and 6 h after the completion of feeding, did not contribute significantly to the total lipid or fatty acid profiles of the *Artemia*. *Artemia* subjected to starvation alone (without beads) failed to evacuate their gut over the 6-h starvation period suggesting they require the intake of suitable-sized particulate matter to undertake gut evacuation. To assess the uptake of EFA in metanauplii (day 2) and juveniles, an enrichment diet containing high levels of arachidonic [AA, 20:4*n*–6] and eicosapentaenoic acid [EPA, 20:5*n*–3] was compared to a basal oat-based diet and a commercial oil emulsion high in docosahexaenoic acid [DHA, 22:6*n*–3]. Both AA and EPA were increased in juvenile *Artemia* within a 24-h enrichment period at a rate proportional to their inclusion in the enrichment, while DHA was incorporated to a lesser degree. For all three EFA, the percentage loss during 6-h starvation was small, but was greater for DHA than EPA or AA. Juvenile *Artemia*, a life stage seldom used in feeding regimes, have the ability to assume the AA and EPA profile of their dietary source. The ability to produce a larger food source with a non-traditional fatty acid profile may be valuable for a number of larval crustacean and fish species.

Keywords: Juvenile *Artemia*, Gut evacuation, Arachidonic acid, Eicosapentaenoic acid, Starvation, Spiny lobster, Phyllosoma

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1. Introduction

Many enrichment protocols for *Artemia* in the past have centered on newly hatched nauplii and in particular, on how to achieve and maintain high levels of docosahexaenoic acid [DHA, 22:6*n*–3], eicosapentaenoic acid [EPA, 20:5*n*–3], and increase the DHA/EPA ratio (Watanabe *et al.*, 1982; Rees *et al.*, 1994; Rasowo *et al.*, 1995; Narciso *et al.*, 1999). While this is a suitable line of investigation for many cultured marine finfish and crustacean species, it may not be suited to phyllosoma larvae of the spiny lobster *Jasus edwardsii* (Phleger *et al.*, 2001), a species under investigation for culture in Australia, New Zealand and Japan. In the wild, phyllosoma larvae of spiny lobster prey on a variety of invertebrates (Shojima, 1963; Thomas, 1963). However, it has been shown that they will reach metamorphosis to puerulus on a diet consisting solely of *Artemia* (Kittaka, 1988; Tong *et al.*, 1997). Optimal growth and survival of phyllosoma are obtained when feeding juvenile *Artemia* (5 day old)

of at least 1.5 mm in length (Tong *et al.*, 1997; Ritar, 2001). It is also likely that they have a requirement for an enrichment product with a greater emphasis on arachidonic acid (AA, 20:4*n*–6) and in particular the EPA/AA ratio (Smith, 1999; Phleger *et al.*, 2001). This is in contrast to current enrichment products which are targeted primarily towards the uptake of DHA and EPA in *Artemia* nauplii and metanauplii (2 day old) (Evjemo *et al.*, 1997; McEvoy & Sargent, 1998; Narciso *et al.*, 1999).

An understanding of the rate of incorporation and loss of essential fatty acids (EFA) in *Artemia* nauplii and metanauplii, during enrichment and subsequent starvation, has assisted in the development of feeding regimes targeted towards finfish (Estévez *et al.*, 1998; Evjemo *et al.*, 2001). While some research has been conducted on enrichment of juvenile *Artemia* (Dhont *et al.*, 1991), there has been little emphasis on AA. The use of enriched juvenile *Artemia* will provide another food source available for crustacean and fish species (Naessens *et al.*, 1997; Smith, 1999; Wouters *et al.*, 1999) while enhancing existing knowledge based primarily on *Artemia* nauplii and metanauplii (Rasowo *et al.*, 1995; Evjemo *et al.*, 1997; Sorgeloos *et al.*, 1998; Han *et al.*, 2000; Evjemo *et al.*, 2001).

A number of larval crustacean species (Abrunhosa & Kittaka, 1997b; Crain, 1999), Nelson & Cox, personal communication) tear large prey organisms into smaller pieces before consuming them, which may result in significant release and loss of material, particularly gut contents, into the surrounding aqueous environment. The benefits of short-term enrichment, where a proportion of the enrichment may reside in the gut of large *Artemia*, with little incorporation into cellular tissue, could be negated with this type of feeding. Our observations (unpublished) suggest that juvenile *Artemia* starved for up to 24 h, failed to fully evacuate their gut cavity. The food was observed as a distinctive coloration, but whether the gut content contributes significantly to the lipid and fatty acid content of the *Artemia* after this period of time is unknown.

The purpose of this study was to determine whether gut content, after short-term enrichment, was an important part of the overall composition of juvenile *Artemia*, and to approximate the fatty acid profile of newly hatched spiny lobster phyllosoma, AA in particular. A novel method is described whereby the food in the gut is evacuated and replaced by inert plastic beads. The target lipid enrichment profiles for *Artemia* were drawn from studies by Smith (1999) and Phleger *et al.* (2001) on the lipid class and fatty acid profiles of spiny lobster phyllosoma.

2. Materials and methods

2.1. Artemia ongrowing

Decapsulated *Artemia* cysts (INVE, Great Salt Lake Prime Gold) were hatched at 28 ± 1°C in 50-l white fibreglass cones in 0.2-μm filtered brackish water (27 ± 1‰). At 24 h, newly hatched *Artemia* nauplii were removed from the hatching containers, rinsed in freshwater for 2 min and transferred to 1000-l conical tanks containing 200 l of filtered seawater (0.2-μm filtered, 34 ± 1‰, 28 ± 1°C). This volume was progressively increased to 1000 l over a 5-day ongrowing period providing a final *Artemia* density of 4 ml^{-1} with a total length of 1.5 ± 0.23 mm (mean ± S.E.M.). During ongrowing for the gut evacuation trial, *Artemia* were fed with an "oat bran diet" consisting (w/w) of 75% oat bran, 12% wheat germ, 7% lecithin and 6% fish oil (Fishaphos, Felton Grimwade and Bickford, Victoria). The fish oil contained (v/v) 17% EPA and 11% DHA. *Artemia* used for the latter enrichment trial received only, oat bran/wheat germ/lecithin (OWL, 50:6:4) during the ongrowing period. *Artemia* diet was added to the water in the 1000-l tanks, three times daily, at a rate to maintain a Secchi depth of 25–30 cm.

2.2. Gut evacuation trial

Juvenile *Artemia* in 1000-l batch cultures were harvested after ongrowing for 5 days. They did not undergo a period of enrichment. An initial sample (0 h) of 32 000 *Artemia* was removed, rinsed, and partitioned into 4 subsamples (fresh and lyophilized samples in dupli-

cate). A further 80 000 *Artemia* were partitioned into either seawater alone without beads (without beads—starvation) or seawater with beads (with beads—forced gut evacuation) at a density of 8 ml^{-1}. The experimental treatments were conducted in white plastic containers holding *Artemia* in 2.5 l of 0.2-μm filtered seawater (28°C and 34 ‰) in duplicate. Individual plastic beads (20–30-μm diameter; Ionics Inc, USA) were suspended in seawater at a density of 1.0×10^6 l^{-1} (beads weighed 4.74 ± 0.19 ng $bead^{-1}$). Counting of beads in the water was undertaken at 0, 3 and 6 h.

2.3. 24-h Artemia enrichment experiment

Three enrichments were used to examine the uptake of essential fatty acids (EFA) in juvenile *Artemia*, and consisted (m/m) of:

1. OWL—oat bran/wheat germ/lecithin (50:6:4)
2. OWL+OIL—oat bran/wheat germ/lecithin/oil (50:6:4:40). The oil consisted (v/v) of a 4.5:1 ratio of Max EPA (containing 18% EPA and 12% DHA, Martek Biosgenes, USA) and ARASCO (containing 40% AA; Martek Biosgenes).
3. A1 DHA Selco (INVE Group, Belgium).

The ratio and level of oil inclusion in enrichment 2 were estimated from examination of oil incorporation in the gut evacuation study. The oat-based diets were prepared daily by blending ingredients suspended in 500 ml of seawater in a household blender (high speed 15 min, Sunbeam, Australia) and large oat particles were removed by sieving (63-μm screen). A1 DHA Selco was prepared according to the manufacturer's directions. All enrichments were applied at a rate of 0.6 g l^{-1}.

As for the gut evacuation trial, the 24-h enrichment experiment utilized *Artemia* ongrown in 1000-l batch culture systems. *Artemia* were harvested on Days 1 and 4 of ongrowing, and partitioned into 1-l beakers at a density of 30 000 *Artemia* metanauplii per beaker and 10000 *Artemia* juveniles per beaker, respectively. Twenty-seven beakers were utilized for each *Artemia* age class; these were divided equally into three groups and enriched with one of three diets for 24 h. After 24 h, triplicate samples for each age class and enrichment were rinsed and stored for analysis (9 beakers per age class). *Artemia* in the remaining beakers were rinsed and resuspended in either seawater alone without beads (without beads) or seawater with beads (with beads), for a further 6 h. After 6 h, all treatments were terminated, rinsed and stored for analyses.

2.4. Lipid class and fatty acid analysis

In general, *Artemia* and feed samples were filtered through 4.7-cm Whatman glass filters (GF/F) and rinsed with 0.5 M ammonium formate. Filters were lyophilized overnight prior to analysis. Dry mass (dm) of the samples ranged from 5.6 to 262.5 mg. Additionally, the method of sample preparation (fresh or lyophilized) was examined to determine whether there was any loss or modification in fatty acid and lipid class composition. At 0 h in the 'gut evacuation trial', duplicate fresh samples were extracted immediately with solvent followed by analysis, while another set of duplicate samples were frozen and lyophilized before extraction.

Samples were quantitatively extracted using a modified Bligh and Dyer (1959) one-phase methanol/chloroform/water extraction (2:1:0.8, by volume); each sample was extracted overnight and the phases were separated the following day by addition of chloroform and water (final solvent ratio, 1:1:0.9, v/v/v, methanol/chloroform/water). The total solvent extract was concentrated (i.e. solvents removed *in vacuo*) using rotary evaporation at 40°C and lipid content determined gravimetrically; weights of lipid were 0.8–49.7 mg. Lipid class analyses were conducted within 3 days, with samples stored in a known volume of chloroform.

An aliquot of the total solvent extract was analyzed using an Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector (TLC–FID) analyzer (Tokyo, Japan) to determine the abundance of individual lipid classes (Volkman & Nichols, 1991). Samples

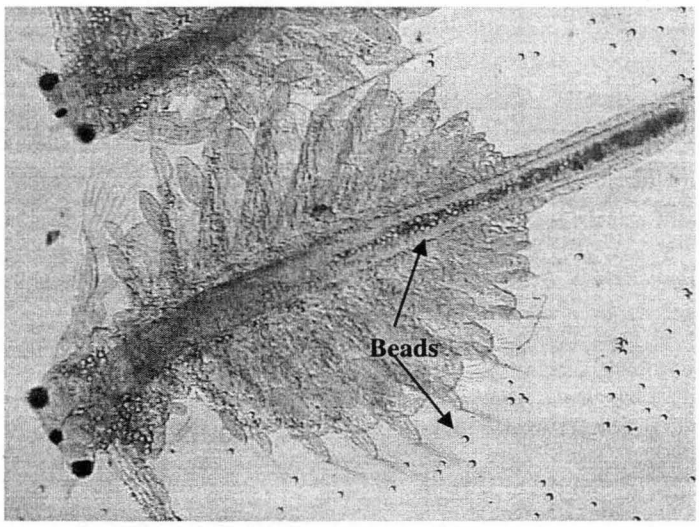

Fig. 1. Within a few minutes of suspending 20–30-μm plastic beads in the water column, they are visible within the gut cavity of *Artemia* due to continuous mechanism of filtration and ingestion.

were applied in duplicate or triplicate to silica gel SIII Chromarods (5-μm particle size) using 1-μl disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The solvent system used for the lipid separation was hexane/diethyl ether/acetic acid (60:17:0.2, by volume), a mobile phase resolving non-polar compounds such as wax ester (WE), triacylglycerol (TAG), free fatty acid (FFA) and sterol (ST). A second non-polar solvent system of hexane/diethyl ether (96:4 v/v) was also used for selected samples to separate hydrocarbon from WE. After development, the chromarods were oven-dried and analyzed immediately to minimize adsorption of atmospheric contaminants. The FID was calibrated for each compound class [phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, WE and TAG (derived from fish oil); 0.1–10-μg range]. Peaks were quantified on an IBM compatible computer using DAPA software (Kalamunda, Western Australia). Iatroscan results are generally reproducible to ± 5% or better for individual classes (Bakes *et al.*, 1995).

Fatty acid methyl esters (FAME) were extracted from an aliquot of the total solvent extract treated with methanol/hydrochloric acid/chloroform (10:1:1, by volume; 80°C, 2 h). FAME were extracted into hexane/chloroform (4:1, v/v, 3 × 1.5 ml) and then treated with *N,O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 50 μl, 60°C, 1 h) to convert sterols to their corresponding trimethylsilyl (TMSi) ethers.

Gas chromatographic (GC) analyses of FAME and sterols were performed with a Hewlett Packard 5890A GC (Avondale, PA) equipped with an HP-5 cross-linked methyl silicone fused silica capillary column (50 m × 0.32 mm i.d.), an FID, a split/splitless injector and an HP 7673A auto sampler. Hydrogen was the carrier gas. Following addition of methyl tricosanoate internal standard, samples were injected in splitless mode at an oven temperature of 50°C. After 1 min, the oven temperature was raised to 150°C at 30°C min^{-1}, then to 250°C at 2°C min^{-1} and finally to 300°C at 5°C min^{-1}. Peaks were quantified with Waters Millennium software (Milford, MA, USA). Individual components were identified using weight spectral data and by comparing retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ± 5% of individual component abundance.

GC–mass spectrometric (GC–MS) analyses were performed on a Thermoquest GCQ GC-mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

Table 1
Lipid class composition (percentage of total lipids), lipid content (wet mass) and percentage composition of major (>2%) and essential fatty acids in juvenile *Artemia* (1.5mm long)

	0 h		3 h		6 h	
	Fresh	Lyophilized	Lyophilized		Lyophilized	
			w/ beads	w/o beads	w/ beads	w/o beads
Lipid composition						
Triacylglycerol	43.8 ± 0.4	44.4 ± 2.6	40.8 ± 1.5	40.9 ± 4.8	43.1 ± 2.1	39.0 ± 2.4
Free fatty acid	1.1 ± 0.2	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	0.9 ± 0.2
Sterol	1.9 ± 0.2	1.9 ± 0.0	1.8 ± 0.7	2.1 ± 0.2	2.1 ± 0.7	1.9 ± 0.2
Polar lipid	53.1 ± 0.8	53.2 ± 2.8	56.7 ± 0.7	56.4 ± 4.4	53.9 ± 3.0	58.2 ± 2.4
Lipid mg g^{-1} (wet mass)	9.4 ± 2.1	7.7 ± 1.5	6.1 ± 1.1	6.1 ± 0.8	5.5 ± 0.1	4.2 ± 1.5
Fatty acid composition[a]						
16:0	12.3 ± 0.2*	11.8 ± 0.0	12.3 ± 0.1	12.2 ± 0.2	12.6 ± 1.0	12.2 ± 0.1
18:2n-6	32.3 ± 0.4*	33.8 ± 0.1	31.6 ± 1.6	32.4 ± 0.2	31.1 ± 0.5	32.5 ± 0.3
18:1n-9c/18.3n-3[b]	27.2 ± 0.5	28.0 ± 0.4	27.0 ± 1.0	27.7 ± 0.3	27.0 ± 0.6	28.0 ± 0.2
18:1n-7c	4.3 ± 0.2	4.4 ± 0.1	4.1 ± 0.3	4.3 ± 0.1	3.9 ± 0.5	4.0 ± 0.3
18:0	6.3 ± 0.1*	5.8 ± 0.1	6.4 ± 0.2	6.4 ± 0.1	7.1 ± 0.5	6.6 ± 0.1
20:4n-6	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.1	0.6 ± 0.0
20:5n-3	3.0 ± 0.0	3.1 ± 0.1	3.0 ± 0.0	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.2
22:6n-3	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0
Other	13.1	13.1	14.1	12.6	13.8	12.2
Sum SFA[c]	21.7 ± 1.0	20.7 ± 0.1	21.5 ± 1.2	21.3 ± 0.8	22.4 ± 1.4	21.1 ± 0.0
Sum MUFA	34.8 ± 0.5	35.5 ± 0.3	34.3 ± 1.4	35.0 ± 0.3	33.8 ± 1.2	34.9 ± 0.2
Sum PUFA	39.8 ± 0.5*	41.4 ± 0.3	40.4 ± 0.0	40.0 ± 0.7	39.6 ± 1.4	40.3 ± 0.4
Sum (n-3)	5.2 ± 0.3	4.9 ± 0.1	5.3 ± 0.2	4.9 ± 0.3	5.4 ± 0.5	5.4 ± 0.5
Sum (n-6)	34.2 ± 0.6*	35.9 ± 0.0	33.8 ± 1.4	34.4 ± 0.4	33.3 ± 1.0	34.2 ± 0.1
Sum (n-3)/(n-6)	0.2	0.1	0.2	0.1	0.2	0.2
Ratio EPA/AA	5.1	5.5	5.4	5.2	5.5	5.3
Ratio DHA/EPA	0.3	0.3	0.3	0.3	0.3	0.3

At time 0 h freshly prepared and lyophilized samples were analyzed. Lyophilized *Artemia* samples were used thereafter at 3 h and 6 h, during forced gut evacuation with beads (w/beads) or starvation without beads (w/o beads). Data are presented as mean ± s.d., $n = 2$.

[a]Other fatty acids at <2%: 14:0, i15:0, 16:1n-9c, 16:1n-7c, C_{16} PUFA, i17:0, 17:0, 18:3n-6, 18:4n-3, 20:1n-11c, 22:0, 22:5n-3 and C22 PUFA.

[b]Under GC these two components coeluted. GC-MS analysis showed that 18:1n-9c was the predominant component.

[c]SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

*Signifies a significant difference between freshly prepared or lyophilized samples at time 0.

2.5. Statistics

Statistical analyses were conducted using one way analysis of variance with Tukey–Kramer HSD tests used for post-hoc comparison (Sokal & Rohlf, 1995). Arcsin$\sqrt{}$ transforms were performed on percentage data. $P < 0.05$ were considered significantly different. Data are presented as mean ± S.D., unless stated. Statistics were executed using JMP version 3.2.1. (SAS Institute).

3. Results

3.1. Gut evacuation trial

3.1.1. Gut evacuation of Artemia with inert plastic beads

Juvenile *Artemia* readily consume plastic beads giving the appearance of a gut cavity full of beads (Fig.1). The mean number of beads counted in the gut cavity of a 1.5-mm, 5-day old juvenile *Artemia*, after 6-h exposure to beads, was 67 ± 6.

At 0 h, there was 1.0×10^6 beads l^{-1} present in the water column. After 3 h, the number of beads suspended in the water column was reduced to $0.533 \times 10^6 \pm 0.012$ beads l^{-1}, which did not differ significantly from the count at 6 h ($0.535 \times 10^6 \pm 0.028$ beads l^{-1}). Uptake of

Table 2
Lipid class composition (percentage of total lipids) and lipid content of enrichments and *Artemia* at Day 1 and Day 4, after 24 h enrichment (Day 2 and Day 5, respectively), and further 6h starvation.

	Wax ester	Triacylglycerol	Free fatty acid	Sterol	Polar lipid	Lipid as mg g^{-1} (dm^1)
Spiny lobster phyllosoma[2]	3.3	2.5	1.3	8 7	81.8	104 6
Enrichments[3]						
OWL	0.0 ± 0.0	33 4 ± 2.3	1 2 ± 0.3	0.7 ± 0.1	64.7 ± 2.5	164.5 ± 1 0
OWL + OIL	0.0 ± 0.0	87.8 ± 1.4	0.0 ± 0.0	1.1 ± 0.2	11.2 ± 1.6	287.8 ± 6 7
A1 DHA Selco	0 2 ± 0 0	85.3 ± 0.2	0 5 ± 0.0	0 5 ± 0.0	13 6 ± 0.1	806.1 ± 38.8
Day 1 *Artemia*	0.6 ± 0 2	[a]45.0 ± 1.4	0.8 ± 0.2	[a]2 3 ± 0 4	[a]51 3 ± 1 9	[a]149 6 ± 5.5
Day 2 *Artemia*						
24h OWL enriched	0.4 ± 0 1 [a]	[a]50.8 ± 3.6[a]	1.7 ± 0.7	[ab]2 8 ± 0.4	[b]44.3 ± 3.4[a]	[a]202.9 ± 37.4
Starved 6h	0 0 ± 0.0 [b]	57 4 ± 2.6[b]	4.1 ± 2.0	2 8 ± 0.6	35.8 ± 2.8[b]	202.2 ± 24.1
Day 2 *Artemia*						
24h OWL + OIL enriched	0 5 ± 0.1[a]	[b]62.1 ± 3.4	3.8 ± 3.2	[a]2.2 ± 0.1	[c]31.5 ± 0 3	[a]203.6 ± 15.1
Starved 6h	0.2 ± 0.1[b]	66 6 ± 3.8	2.1 ± 1.5	2 1 ± 0.4	28 9 ± 3 2	264.3 ± 91.8
Day 2 *Artemia*						
24h DHA enriched	0.7 ± 0.1 [a]	[b]65.3 ± 1.5	5.0 ± 0.3 [a]	[b]3.1 ± 0.2[a]	[c]26.0 ± 1.5	[b]264.7 ± 17.3
Starved 6h	0.2 ± 0.2[b]	73 2 ± 9 4	1.5 ± 0.4[b]	1.6 ± 0.6[b]	23.4 ± 8 4	255 2 ± 28.8
Day 4 *Artemia*	0.3 ± 0.2	[a]40 7 ± 1.7	[a]1 2 ± 0.5	[a]3 4 ± 0 3	[a]54 5 ± 2.2	[a]182 1 ± 34.3
Day 5 *Artemia*						
24h OWL enriched	0.2 ± 0.2	[b]68.4 ± 13.6	[a]1.6 ± 1.0	[a]1 9 ± 0.9	[b]27.9 ± 11 7	[a]186.4 ± 7.3
Starved 6h	0.1 ± 0.1	46.6 ± 19.3	2.8 ± 0.9	3.0 ± 0.6	47.5 ± 18 7	151.0 ± 20.1
Day 5 *Artemia*						
24h OWL + OIL enriched	0.1 ± 0 2	[b]70.6 ± 1 9	[ab]1.8 ± 0 7	[ab]2.2 ± 0.4	[b]25.3 ± 1.8	[b]258 9 ± 5.8
Starved 6h	0 1 ± 0.1	69.2 ± 2 6	3 4 ± 2.3	1.8 ± 0.2	25.6 ± 0 8	242.6 ± 74.7
Day 5 *Artemia*						
24h DHA enriched	0.0 ± 0 0	[b]71.8 ± 3.3	[b]4.4 ± 1.5[a]	[b]1 6 ± 0.5	[b]22 3 ± 1 3	[b]296 5 ± 31.7
Starved 6h	0.0 ± 0.0	74 8 ± 2.7	1 9 ± 0.7[b]	1.2 ± 0.1	22 1 ± 2 7	283.8 ± 34.8

Newly-hatched spiny lobster phyllosoma profiles (target predator species) are included for comparison (Smith,1999)
Data are presented as mean ± s d. (n = 3, except for starved 6 h, where n = 6). Different superscript preceding data values denote significant differences between Day 1 or Day 4 ongrown Artemia and Artemia after 24h enrichment
Different superscripts following data values denote significant differences between 24 h enrichment and 6 h starvation within a dietary group.
[1]dm = dry mass
[2]In phyllosoma samples, wax ester also contains hydrocarbon; sterol contains diacylglycerol.
[3]Enrichment composition: OWL - oat bran wheat germ lecithin (50 6 4), OWL+OIL - oat bran: wheat germ. lecithin: oil (50:6.4:40). Oil composed of Max EPA ARASCO - 4 5:1, A1 DHA Selco (INVE Group, Belgium)

beads into the *Artemia* was therefore approximately 50% of the available beads in the water column at 3 and 6 h. More than 50% of the beads in the water column at 3 h were fouled with faecal material indicating the passage of beads through the *Artemia* took less than 3 h. The distinctive brown-yellow coloration previously evident in the gut of all *Artemia* was reduced to negligible levels (visual observation) in the with beads treatment after 6 h, but not at 3 h. In *Artemia* in the without beads treatment, there was no visible reduction in the gut coloration at 3 or 6 h compared to 0 h. Only a minor amount of faecal material was detected in the water column during the 6 h starvation period (*Artemia* without beads), indicating that only minimal voiding of the gut contents into the water column had occurred in this treatment.

3.1.2. Juvenile Artemia - lipid class and fatty acid composition

The method of sample preparation (freshly prepared or lyophilized) did not significantly alter the lipid content nor the percent of lipid class extracted (9.4 ± 2.1 mg g^{-1} wet mass (wm) compared to 7.7 ± 1.5 mg g^{-1}wm, respectively, Table 1). Due to the method of sample preparation where freshly prepared samples were not dried before extraction, only wet mass is presented in this section. Although a decrease in lipid content was observed in *Artemia* between 0, 3 and 6 h, this was not significant. There was no difference in the lipid class composition in the treatments, with or without beads at different sampling times. Polar lipid (PL) was the major lipid class (LC), comprising 53–58% of total lipid, followed by TAG,

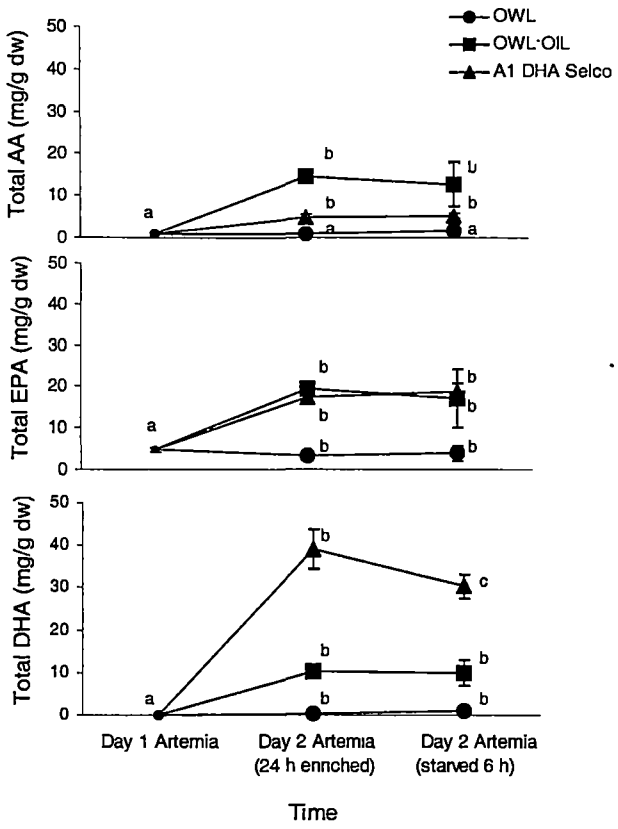

Fig. 2. Changes in the quantitative contribution of arachidonic (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to the total lipid in Day 1 *Artemia*, after 24-h enrichment (Day 2) and a further 6-h starvation. Different superscripts denote significant differences within a dietary treatment over time.

with 39–44% of total lipid. All samples contained low levels of sterol (ST) (1.8–2.1%), and free fatty acid (FFA) (0.5–1.1%).

There were only minor significant differences between the fatty acid profiles of freshly extracted and lyophilized *Artemia*, and did not include the EFA (Table 1). It was therefore considered that either method was appropriate for subsequent analyses, lyophilized samples were used thereafter. There were no significant differences in the fatty acid profiles of any of the lyophilized samples across all sampling times either w/beads or w/o beads. The essential fatty acids, AA, EPA and DHA, were present as minor components (0.6–3.1%) of the total fatty acid profile.

3.2. 24-h Artemia enrichment experiment

3.2.1. Artemia size

There was a significant increase in *Artemia* length following 24-h enrichment for each age class, but there was no significant difference between enrichment treatments. *Artemia* total length at Days 1 and 4 was 0.72 ± 0.01 and 1.38 ± 0.01 mm, respectively, and following 24-h enrichment, total length increased to 0.82 ± 0.02 and 1.49 ± 0.03 mm, respectively (n = 15 for all *Artemia* measures).

3.2.2. Dietary lipid and fatty acid composition

The major lipid classes in the enrichment diets were TAG and PL (33–88% and 11–65% of total lipid, respectively; Table 2). ST and FFA were approximately 1% or less, and wax ester (WE) was only detected in the A1 DHA Selco diet (0.2%). The OWL+OIL and A1 DHA Selco diets were high in lipid (288 and 806 mg g^{-1} dry mass (dm), respectively) domi-

nated by TAG (85 and 88%, respectively), in contrast to the PL-dominated (65%) OWL enrichment containing 165 mg g^{-1} dm of lipid. The dominant fatty acids in the OWL enrichment diet (Table 3), were 18:1*n*–9, 18:2*n*–6 and 16:0, while AA and EPA made substantial contributions to the OWL+OIL diet (8.9% and 11.7% respectively, Table 4), as did DHA to the A1 DHA Selco diet (20.7%, Table 5). The latter two diets contained substantially lower levels of 18:2*n*–6 and 18:1*n*–9 compared to that found in the OWL diet. The *n*–3/*n*–6 ratio of the three enrichment diets differed substantially. The OWL diet was dominated by high levels of *n*–6 fatty acids (*n*–3/*n*–6 ratio of <0.1), whereas equal amounts of *n*–3 and *n*–6 fatty acids were present in the OWL+ OIL diet (1.1), and the *n*–3 class dominated the A1 DHA Selco diet (*n*–3/*n*–6 ratio of 3.1). The ratios of the *n*–3/*n*–6 fatty acids in the different diets was mirrored by their respective EPA/AA ratios.

3.2.3. Enriched Artemia - lipid and fatty acid composition

There were no significant differences across all enrichment treatments and *Artemia* age in the lipid class profiles for animals starved w/beads or w/o beads, so data from both starved treatments was pooled (n = 6) for comparisons with 24-h enrichment (Table 2). Lipid data for Stage I spiny lobster phyllosoma (obtained from wild caught ovigerous females), the target predator species are also included for comparison. As was seen in the enrichment profiles, TAG and PL in 24-h enriched *Artemia* (Days 2 and 5) were the dominant lipid classes. There were significant increases in the relative level of TAG in *Artemia* exposed to all enrichments after 24-h. The greatest increases were present in *Artemia* fed the OWL+OIL (38 and 73% Days 2 and 5, respectively) and A1 DHA Selco (41 and 76% Day 2s and 5, respectively) diets. As a result of the increased TAG, the percentage contribution of PL was significantly reduced in all 24-h enriched *Artemia*. All Day 5 *Artemia* contained between 7–18% more TAG than Day 2 *Artemia*.

After 24-h enrichment and with 6-h starvation, Day 2 OWL-enriched *Artemia* demonstrated a further significant increase in TAG, while PL decreased. No such changes were evident in TAG or PL in any of the other treatments at Day 2 or 5. A number of small but significant shifts in the minor lipid components (WE, FFA and ST) occurred in all three enrichment treatments after 6-h starvation. However, this was confined to Day 2 *Artemia*, and the FFA levels of A1 DHA Selco-enriched Day 5 *Artemia*.

In Day 2 *Artemia*, the amount of lipid accumulated expressed as mg g^{-1} dm was significantly greater in animals enriched for 24-h with A1 DHA Selco, while in Day 5 *Artemia* there were significant increases in lipid dry mass in both OWL+OIL and A1 DHA Selco-enriched animals. There was no significant change in lipid dry mass across any treatment or *Artemia* age with starvation.

The major fatty acids in Day 1 and Day 4 *Artemia* were 18:1*n*–9, 18:2*n*–6, 16:0, 18:1*n*–7, 18:0 (Tables 3–5). A number of these fatty acids were also prominent in the diets, and subsequently, in the 24-h enriched Day 2 and Day 5 *Artemia*. In particular, 18:2*n*–6 made a large contribution to the total fatty acid profile of the OWL diet (44.4%) with subsequent transfer to both Day 2 and 5 *Artemia*. The OWL+OIL and A1 DHA Selco diets contained lower levels of 18:2*n*–6 (10.0 and 5.4%, respectively). However, in both cases 18:2*n*–6 still assumed major prominence in 24-h enriched *Artemia* (range of 8.9–10.3% in Day 2 *Artemia* and 13.2–16.9% in Day 5 *Artemia*).

OWL+OIL and A1 DHA Selco-enriched *Artemia* incorporated increased proportions of EFA largely reflecting the profiles of their diets. There was a greater uptake of AA and EPA seen in Day 5 *Artemia* compared to Day 2 *Artemia*, while the uptake of DHA was similar for both Day 2 and Day 5 *Artemia*. Where these three EFA were present as minor enrichment components, such as in the OWL diet (0.1%), enrichment resulted in EFA depletion in Day 5 enriched *Artemia*. When the dietary contribution of AA, EPA and DHA to Day 5 *Artemia* was prominent (OWL+OIL—8.9%, 11.7%, 7.5%, respectively; A1 DHA Selco—1.7%, 5.3%, 20.7%, respectively), starvation caused a reduction in their percentage contribution, and in particular, the contribution of the essential fatty acid DHA.

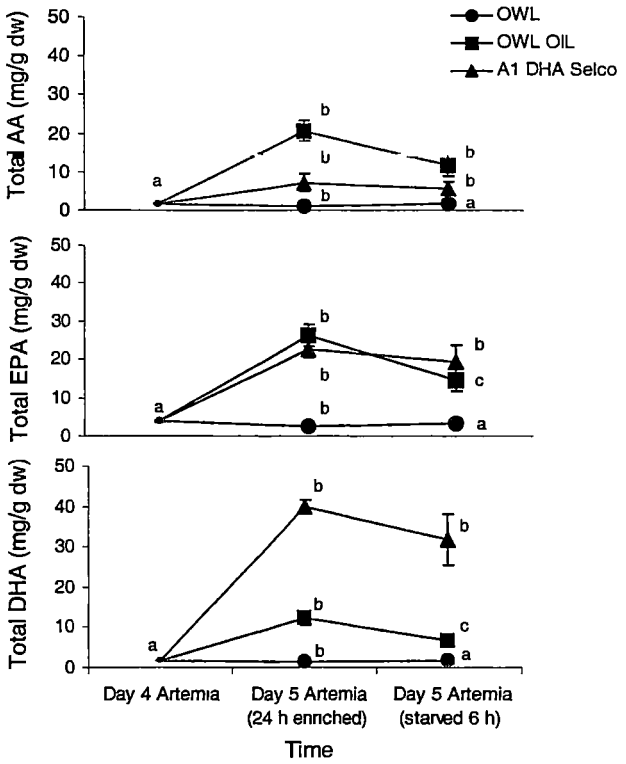

Fig. 3. Changes in the quantitative contribution of arachidonic (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to the total lipid in Day 4 *Artemia*, after 24-h enrichment (Day 5) and a further 6 h starvation. Different superscripts denote significant differences within a dietary treatment over time.

The quantitative changes in the EFA (Figs. 2 and 3) largely mirrored the results of the percentage data (Tables 3–5). An exception was evident in the essential fatty acid DHA in the A1 DHA Selco treatment, where Day 2 and Day 5 starved *Artemia* lost 22% ($P < 0.05$, Fig. 2) and 20% ($P = 0.07$, Fig. 3), respectively, compared to pre-starvation levels.

Other fatty acids reduced by starvation (Tables 3–5) included 18:1*n*–7 and 18:2*n*–6, while the saturated fatty acids (SFA), 16:0 and 18:0, underwent a degree of sparing with starvation. The large contribution of 18:2*n*–6 to the diets and subsequently the *Artemia* is reflected in low *n*–3/*n*–6 ratios. In particular, the *n*–3/*n*–6 ratio in the OWL and OWL+OIL-fed animals was in the range of 0.1–0.7 and 0.3–1.0, respectively, compared to the target phyllosoma ratio of 2.7.

4. Discussion

4.1. Gut evacuation trial

4.1.1. Gut evacuation

Even though enrichment of *Artemia* is widely used in aquaculture, it has, to date, been unclear what proportion of the enrichment is incorporated into body tissue and how much remains resident in the gut. The gut evacuation trial demonstrated that the gut content of juvenile *Artemia* (5 day old) at 3 h and 6 h made only a minor contribution to the total lipid and fatty acid profiles of the *Artemia*. While minimal gut retention times were not monitored in this trial, we have previously recorded a gut retention time of 7 min for actively feeding juvenile *Artemia* (Smith, unpublished). This is intermediate to retention times previously recorded for nauplii and adult *Artemia* of 10 and 3 min, respectively (Dobbeleir *et al.*, 1980). While retention times in juvenile *Artemia* of up to 30 min have been recorded using starch to replace gut content (Nimura, 1989) we considered this time period is a meas-

ure of the time required to flush the alimentary canal of a particular material rather than gut passage time. In our trial, flushing of gut coloration in *Artemia* using beads was not achieved by 3 h and completed by 6 h. The reduction in gut coloration was thought to be due to sequential loss of bulk food content and then associated pigmentation from the gut lining, it is thought the time required for flushing differed from Nimura (1989) due to the different materials used for gut evacuation. In the w/o beads *Artemia* samples, there was no visible difference between the gut content prior to and after 6-h starvation with minimal faeces present in the water column. *Artemia* are continuous, non-selective filter feeders (Provasoli & Shiraishi, 1959; Sorgeloos *et al.*, 1998), so it appears that without the intake of suitable sized particulate matter the normal processes to stimulate gut evacuation do not occur. Material is maintained in the gut for at least 6 h when *Artemia* are held at 28°C in clear water with no particulate matter present.

4.1.2. Juvenile Artemia - lipid and fatty acid composition

Artemia in the without beads treatment still had food remaining in their gut for at least 6 h after the commencement of the trial, i.e., after feeding was ceased. It is probable that this prolonged gut resident time would allow the extraction of additional lipid from the food than otherwise would have occurred in actively feeding *Artemia.* As there was no difference in the amount of lipid between evacuated *Artemia* (with beads) and starved *Artemia* (without beads) at 3 h, we propose that the majority of the lipid was removed by 3 h with resident material in the gut devoid of lipids. Therefore, any loss of gut content through predator feeding behavior, as often occurs when *Artemia* are fed to spiny lobster phyllosoma, should only alter the total *Artemia* lipid profile within a short period of the commencement of feeding, i.e. <3 h, after which time the remaining lipid in the gut content can be considered as largely not contributing to the juvenile *Artemia* lipid profile. We found, as did Léger *et al.* (1986; 1987b), that the fatty acid profile of unenriched *Artemia* contains only small amounts of the essential fatty acids AA, EPA and DHA (see Table 1). This finding highlights the need for a suitable source of enrichment if *Artemia* were to match the observed lipid profiles for spiny lobster phyllosoma (Smith, 1999; Phleger *et al.*, 2001).

4.2. 24-h Artemia enrichment experiment

4.2.1. Diets and Artemia - lipid and fatty acid composition

The 24-h enrichment trial reinforced the minor role that the gut content had on the total lipid or fatty acid profile of Day 5 *Artemia.* The result of minimal influence of gut content on lipid or fatty acid profiles was confirmed for Day 2 *Artemia* when sampled 6 h later. The enrichment diets used in the 24-h enrichment trial differed substantially from each other in both the level and type of lipid inclusion. Prior to enrichment, PL was the dominant lipid class in both Day 1 and Day 4 *Artemia.* However, as seen in other trials after enrichment, TAG became the major lipid class (McEvoy *et al.*, 1996), even in *Artemia* fed a PL-rich diet (OWL). This is probably due to *Artemia* taking up lipid in excess to requirements and storing it as TAG, a readily available energy source (Wickins *et al.*, 1995). The efficiency at which lipids were assimilated varied greatly between diets. A1 DHA Selco is composed of 806 mg g^{-1} lipid dm, 4.9 and 2.8 times greater than the lipid content of the OWL and OWL+OIL diets, respectively. While A1 DHA Selco provided significantly greater lipid inclusion to Day 2 *Artemia* than did the enrichments of OWL and OWL+OIL, this pattern was not repeated for larger juvenile *Artemia.* There was no significant difference between the lipid dm of Day 5 *Artemia* enriched with either A1 DHA Selco or OWL+ OIL regardless of whether the enrichment contained a high lipid content (A1 DHA Selco, 81% lipid) compared to one with a moderate level of lipid (OWL+ OIL, 29% lipid). This absence of a difference suggests that juvenile *Artemia* have a maximum lipid uptake during the 24-h enrichment period. In juvenile *Artemia,* the low uptake rate of enrichment products that are high in lipid suggests that increasing the density of *Artemia* in the enrichment diet, or reducing the application rate of the lipid emulsion would not appreciably alter lipid uptake.

Table 3
Percentage composition of major (>2%) and essential fatty acids in OWL enrichment, *Artemia* at Day 1 and Day 4, after 24 h enrichment with OWL (Day 2 and Day 5, respectively) and 6h starvation

	spiny lobster phyllosoma	OWL enrichment	Ongrown Day 1 *Artemia*	24h enrichment (Day 2 *Artemia*)	6h starved (Day 2 *Artemia*)	Ongrown Day 4 *Artemia*	24h enrichment (Day 5 *Artemia*)	6 h starved (Day 5 *Artemia*)
14:0	0.9	0.1 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.2	1.8 ± 0.0^{a}	1.0 ± 0.1^{b}	1.2 ± 0.2^{b}
16:1n-7*c*	2.3	0.1 ± 0.0	3.2 ± 0.2^{a}	2.2 ± 0.0^{b}	2.3 ± 0.2^{b}	2.8 ± 0.0^{a}	1.5 ± 0.1^{c}	2.0 ± 0.1^{b}
16:0	13.1	19.1 ± 0.1	11.6 ± 0.1^{b}	11.0 ± 0.4^{b}	12.3 ± 0.5^{a}	12.3 ± 0.3^{a}	11.5 ± 0.2^{b}	12.5 ± 0.2^{a}
18:4n-3	0.0	0.0 ± 0.0	3.7 ± 0.1^{a}	1.7 ± 0.1^{b}	1.5 ± 0.2^{b}	3.5 ± 0.2^{a}	1.3 ± 0.0^{c}	1.7 ± 0.1^{b}
18:2n-6	1.8	44.4 ± 0.5	10.3 ± 0.3^{b}	25.2 ± 0.4^{a}	25.0 ± 1.3^{a}	22.8 ± 0.1^{c}	35.2 ± 0.7^{a}	30.6 ± 0.8^{b}
18:1n-9c/18:3n-3 [1]	13.2	30.1 ± 0.3	42.9 ± 0.6^{a}	36.5 ± 1.1^{b}	35.6 ± 1.3^{b}	29.5 ± 0.4^{b}	31.2 ± 0.8^{ab}	29.8 ± 1.2^{b}
18:1n-7*c*	4.8	1.2 ± 0.0	8.4 ± 0.2^{a}	6.3 ± 0.2^{b}	5.7 ± 0.4^{c}	5.9 ± 0.1^{a}	3.7 ± 0.1^{b}	4.3 ± 0.2^{b}
18:0	7.2	2.6 ± 0.0	5.8 ± 0.1^{b}	6.1 ± 0.4^{a}	6.2 ± 0.2^{a}	6.9 ± 0.0^{a}	5.5 ± 0.0^{b}	6.6 ± 0.2^{a}
20:4n-6	8.2	0.1 ± 0.0	0.6 ± 0.0^{b}	0.6 ± 0.1^{ab}	1.1 ± 0.8^{a}	1.2 ± 0.0^{a}	0.7 ± 0.0^{b}	1.2 ± 0.2^{b}
20:5n-3	20.3	0.1 ± 0.0	3.1 ± 0.0^{a}	2.0 ± 0.0^{b}	2.5 ± 0.9^{b}	2.8 ± 0.0^{a}	1.6 ± 0.1^{b}	2.5 ± 0.3^{a}
22:6n-3	11.3	0.0 ± 0.0	0.0 ± 0.0^{b}	0.3 ± 0.2^{a}	0.6 ± 0.5^{a}	1.2 ± 0.0^{a}	0.7 ± 0.1^{b}	1.1 ± 0.2^{ab}
Other[2]	16.9	2.2	9.6	7.3	6.4	9.3	6.1	6.5
Sum (n-3)	33.1	0.1 ± 0.0	7.5 ± 0.1^{a}	4.3 ± 0.2^{b}	4.8 ± 1.6^{b}	8.0 ± 0.3^{a}	3.8 ± 0.1^{c}	5.5 ± 0.6^{b}
Sum (n-6)	12.2	44.6 ± 0.4	11.5 ± 0.2^{b}	26.3 ± 0.6^{a}	26.6 ± 0.9^{a}	25.2 ± 0.1^{c}	36.6 ± 0.8^{a}	32.7 ± 0.6^{b}
Sum (n-3)/(n-6)	2.7	<0.1	0.7^{a}	0.2^{b}	0.2^{b}	0.3^{a}	0.1^{c}	0.2^{b}
Ratio EPA/AA	2.5	0.7	5.0^{a}	3.6^{b}	2.9^{b}	2.3^{a}	2.2^{b}	2.0^{b}
Ratio DHA/EPA	0.6	0.5	0.0^{b}	0.1^{a}	0.2^{a}	0.4	0.5	0.4
Total FA (mg g^{-1})			144.3 ± 10.6	167.5 ± 7.0	150.8 ± 19.4	138.4 ± 7.4	165.0 ± 14.0	139.5 ± 21.3

Newly-hatched spiny lobster phyllosoma profiles (target predator) are included for comparison (Smith 1999). Data are presented as mean ± s.d. (n = 3, except for starved 6 h, where n = 6). Enrichment composition: OWL - oat bran: wheat germ: lecithin (50:6:4). Different superscript denotes a significant difference between Day 1 or Day 5 *Artemia*, 24h enrichment and 6h starvation.

[1]Under GC, these two components coeluted. GC-MS analysis showed that 18:1n-9*c* was the predominant component.

[2]Other fatty acids include: i15:0, 16:1n-9c, C_{16} PUFA, i17:0, 17:0, 18:3n-6, 20:1n-11*c*, 22:0, 22:5n-3 and C_{22} PUFA.

Table 4
Percentage composition of major (>2%) and essential fatty acids in OWL+OIL enrichment, *Artemia* at Day 1 and Day 4, after 24 h enrichment with OWL+ OIL (Day 2 and Day 5, respectively) and 6h starvation

	spiny lobster phyllosoma	OWL + OIL enrichment	Ongrown (Day 1 *Artemia*)	24h enrichment (Day 2 *Artemia*)	6h starved (Day 2 *Artemia*)	Ongrown (Day 4 *Artemia*)	24h enrichment (Day 5 *Artemia*)	6 h starved (Day 5 *Artemia*)
14:0	0.9	5.6 ± 0.2	0.8 ± 0.0^{b}	2.1 ± 0.0^{a}	2.0 ± 0.5^{a}	1.8 ± 0.0^{b}	2.2 ± 0.0^{a}	2.3 ± 0.2^{a}
16:1n-7*c*	2.3	6.6 ± 0.6	3.2 ± 0.2^{b}	4.3 ± 0.3^{a}	4.6 ± 0.5^{a}	2.8 ± 0.0^{b}	4.8 ± 0.1^{a}	4.8 ± 0.2^{a}
16:0	13.1	17.1 ± 0.4	11.6 ± 0.1	11.7 ± 0.4	11.5 ± 1.6	12.3 ± 0.3^{a}	10.3 ± 0.1^{b}	11.9 ± 0.4^{a}
18:4n-3	0.0	0.0 ± 0.0	3.7 ± 0.1^{a}	1.9 ± 0.1^{b}	2.1 ± 0.2^{b}	3.5 ± 0.2^{a}	2.1 ± 0.1^{b}	2.0 ± 0.2^{b}
18:2n-6	1.8	10.0 ± 0.3	10.3 ± 0.3	10.3 ± 0.6	11.8 ± 1.1	22.8 ± 0.1^{a}	16.7 ± 0.2^{b}	16.9 ± 1.1^{b}
18:1n-9c/18:3n-3[1]	13.2	14.2 ± 0.2	42.9 ± 0.6^{a}	26.8 ± 0.3^{b}	28.3 ± 1.0^{b}	29.5 ± 0.4^{a}	22.2 ± 0.3^{b}	21.7 ± 4.1^{b}
18:1n-7*c*	4.8	2.8 ± 0.0	8.4 ± 0.2^{a}	6.0 ± 0.2^{b}	6.3 ± 0.2^{b}	5.9 ± 0.1^{a}	4.1 ± 0.1^{c}	5.0 ± 0.3^{b}
18:0	7.2	4.4 ± 0.1	5.8 ± 0.1^{a}	5.3 ± 0.3^{b}	5.3 ± 0.4^{ab}	6.9 ± 0.0^{a}	4.1 ± 0.0^{c}	5.3 ± 0.3^{b}
20:4n-6	8.2	8.9 ± 0.0	0.6 ± 0.0^{b}	7.4 ± 0.6^{a}	6.0 ± 2.5^{a}	1.2 ± 0.0^{c}	8.9 ± 0.2^{a}	7.5 ± 0.9^{b}
20:5n-3	20.3	11.7 ± 0.3	3.1 ± 0.0^{b}	9.9 ± 0.3^{a}	8.1 ± 3.2^{a}	2.8 ± 0.0^{c}	11.3 ± 0.1^{a}	9.5 ± 0.8^{b}
22:6n-3	11.3	7.5 ± 0.0	0.0 ± 0.0^{b}	5.2 ± 0.2^{a}	4.5 ± 1.1^{a}	1.2 ± 0.0^{c}	5.3 ± 0.4^{a}	4.3 ± 0.6^{b}
Other[2]	16.9	11.2	9.6	9.1	9.5	9.3	8.0	8.8
Sum (n-3)	33.1	23.0 ± 0.4	7.5 ± 0.1^{b}	18.7 ± 0.4^{a}	16.3 ± 2.2^{a}	8.0 ± 0.3^{c}	20.3 ± 0.5^{a}	17.2 ± 1.7^{b}
Sum (n-6)	12.2	20.5 ± 0.5	11.5 ± 0.2^{b}	19.0 ± 0.2^{a}	19.2 ± 2.2^{a}	25.2 ± 0.1	27.2 ± 0.2	25.9 ± 1.6
Sum (n-3)/(n-6)	2.7	1.1	0.7^{b}	1.0^{a}	0.8^{b}	0.3^{c}	0.7^{a}	0.7^{b}
Ratio EPA/AA	2.5	1.3	5.0^{a}	1.3^{b}	1.4^{b}	2.3^{a}	1.3^{b}	1.3^{b}
Ratio DHA/EPA	0.6	0.6	0.0^{b}	0.5^{a}	1.1^{a}	0.4	0.5	0.4
Total FA (mg g^{-1})			144.3 ± 10.6^{a}	197.7 ± 10.2^{b}	214.5 ± 12.2^{b}	138.4 ± 7.4^{a}	231.8 ± 26.0^{b}	139.5 ± 21.3^{a}

Newly-hatched spiny lobster phyllosoma profiles (target predator species) are included for comparison (Smith, 1999). Data are presented as mean ± s.d. (n = 3, except for starved 6 h, where n = 6). Enrichment composition: OWL+OIL - oat bran: wheat germ: lecithin: oil (50:6:4:40). Oil composed of Max EPA: ARASCO - 4.5:1. See Table 3 for additional information.

Table 5
Percentage composition of major (>2%) and essential fatty acids in A1 DHA Selco enrichment, *Artemia* at Day 1 and Day 4, after 24 h enrichment with A1 DHA Selco (Day 2 and Day 5, respectively) and 6h starvation

	J. edwardsii phyllosoma	A1 DHA Selco enrichment	Ongrown (Day 1 *Artemia*)	24h enrichment (Day 2 *Artemia*)	6h starved (Day 2 *Artemia*)	Ongrown (Day 4 *Artemia*)	24h enrichment (Day 5 *Artemia*)	6 h starved (Day 5 *Artemia*)
14:0	0.9	3.3 ± 0.0	0.8 ± 0.0^{c}	1.5 ± 0.0^{a}	1.2 ± 0.1^{b}	1.8 ± 0.0	1.6 ± 0.1	1.5 ± 0.1
16:1n-7*c*	2.3	4.2 ± 0.2	3.2 ± 0.2^{b}	3.6 ± 0.1^{b}	4.0 ± 0.1^{a}	2.8 ± 0.0^{c}	4.1 ± 0.1^{b}	4.4 ± 0.2^{a}
16:0	13.1	21.4 ± 0.6	11.6 ± 0.1^{c}	12.7 ± 0.4^{a}	10.7 ± 0.4^{b}	12.3 ± 0.3^{a}	11.0 ± 0.2^{b}	10.6 ± 0.3^{b}
18:4n-3	0.0	0.0 ± 0.0	3.7 ± 0.1^{a}	1.3 ± 0.0^{c}	1.4 ± 0.0^{b}	3.5 ± 0.2^{a}	1.6 ± 0.1^{b}	1.6 ± 0.1^{b}
18:2n-6	1.8	5.4 ± 0.2	10.3 ± 0.3^{ab}	8.9 ± 0.3^{b}	10.2 ± 0.6^{a}	22.8 ± 0.1^{a}	13.2 ± 0.1^{b}	14.2 ± 0.6^{b}
18:1n-9c/18:3n-3[1]	13.2	18.0 ± 3.0	42.9 ± 0.6^{a}	27.9 ± 0.1^{c}	30.6 ± 1.0^{b}	29.5 ± 0.4^{a}	24.0 ± 0.1^{b}	24.9 ± 0.8^{b}
18:1n-7*c*	4.8	3.0 ± 0.2	8.4 ± 0.2^{a}	5.6 ± 0.1^{c}	6.2 ± 0.3^{b}	5.9 ± 0.1^{a}	4.7 ± 0.2^{c}	5.0 ± 0.1^{b}
18:0	7.2	6.0 ± 0.2	5.8 ± 0.1^{a}	4.7 ± 0.2^{a}	4.3 ± 0.1^{b}	6.9 ± 0.0^{a}	4.2 ± 0.1^{b}	4.1 ± 0.2^{b}
20:4n-6	8.2	1.7 ± 0.1	0.6 ± 0.0	1.9 ± 0.0	2.1 ± 0.2	1.2 ± 0.0^{b}	2.7 ± 0.7^{a}	2.4 ± 0.2^{a}
20:5n-3	20.3	5.3 ± 0.3	3.1 ± 0.0^{b}	7.1 ± 0.1^{a}	7.7 ± 0.2^{a}	2.8 ± 0.0^{b}	8.7 ± 0.3^{a}	8.2 ± 0.3^{a}
22:6n-3	11.3	20.7 ± 0.6	0.0 ± 0.0^{c}	15.8 ± 0.3^{a}	12.4 ± 0.4^{b}	1.2 ± 0.0^{c}	15.4 ± 1.1^{a}	13.6 ± 0.7^{b}
Other[2]	16.9	11.0	9.6	9.0	9.2	9.3	8.8	9.5
Sum (n-3)	33.1	28.1 ± 1.1	7.5 ± 0.1^{b}	25.6 ± 0.4^{a}	22.8 ± 0.7^{a}	8.0 ± 0.3^{c}	27.0 ± 0.8^{a}	24.6 ± 0.8^{b}
Sum (n-6)	12.2	9.2 ± 0.4	11.5 ± 0.2^{a}	12.4 ± 0.3^{b}	13.9 ± 0.4^{b}	25.2 ± 0.1^{a}	17.8 ± 0.7^{b}	18.4 ± 0.8^{b}
Sum (n-3)/(n-6)	2.7	3.1	0.7 ^{a}	2.1 ^{b}	1.6 ^{b}	0.3 ^{c}	1.5 ^{a}	1.3 ^{b}
Ratio EPA/AA	2.5	3.2	5.0 ^{a}	3.7 ^{b}	3.6 ^{b}	2.3 ^{b}	3.2 ^{a}	3.4 ^{a}
Ratio DHA/EPA	0.6	3.9	0.0 ^{a}	2.2 ^{b}	1.6 ^{b}	0.4 ^{b}	1.8 ^{a}	1.6 ^{a}
Total FA (mg g^{-1})			144.3 ± 10.6^{a}	247.0 ± 31.0^{b}	245.1 ± 18.9^{b}	138.4 ± 7.4^{a}	261.0 ± 17.7^{b}	236.2 ± 54.1^{b}

Newly-hatched spiny lobster phyllosoma profiles (target predator) are included for comparison (Smith, 1999). Data are presented as mean ± s.d. (n = 3, except for starved 6 h, where n = 6). Enrichment composition: A1 DHA Selco (INVE Group, Belgium). See Table 3 for additional information.

Starvation for 6 h at 28°C in this study resulted in no observable difference in lipid content in 2- or 5-day old *Artemia*, although there were significant changes in fatty acids. This contrasts with the findings of Evjemo *et al.* (2001), who found that *Artemia* nauplii lost 34% of their lipid content during 24-h starvation at 26°C, while at 12°C lipid losses during starvation were reduced to 11%. We suggest that during the initial 6 h of starvation lipid was conserved at the expense of another metabolic substrate, possibly carbohydrate, which has been demonstrated as important energy source in juvenile and adult *Artemia* (D'Agostino, 1980). A reduction in the abundance of some fatty acids such as, AA and EPA in the OWL+OIL treatment suggest they were supplied to *Artemia* in excess of their requirement, or as in the case of DHA whereby *Artemia* find it difficult to maintain as a storage product (Evjemo *et al.*, 2001). As it is the intention to feed enriched juvenile *Artemia* to the predator spiny lobster phyllosoma at 18°C (Ritar, 2001), the loss of lipids in enriched juvenile *Artemia* during a 20-h tank resident time would be low. Our results suggest that while there would be a decline in the essential fatty acid DHA over time in juvenile *Artemia*, it would occur at a marginally slower rate than if Day 2 metanauplii *Artemia* (this trial) or nauplii (Evjemo *et al.*, 2001) were used. The large contribution of 18:2*n*–6 to the total fatty acid profile of both diet and enriched *Artemia* resulted in a low *n*–3/*n*–6 ratio, particularly in the OWL and to a lesser extent the OWL+OIL fed animals. We suggest that feeding these *Artemia* may result in the transfer of a low *n*–3/*n*–6 ratio to the predator species. It has been postulated, particularly in fish, that *n*–3/*n*–6 ratios less than 1, as occurs in the OWL and OWL+OIL enriched *Artemia*, may increase larval susceptibility to stress (Sargent, 1995). By reducing the level of 18:2*n*–6 in the *Artemia* diet (hence in *Artemia*), the *n*–3/*n*–6 ratio potentially could be increased. This would be at the expense of the potential benefits of 18:2*n*–6 being available as an energy source (D'Souza, 1998) or precursor for AA production (Sargent, 1995). However, this should not be considered a negative factor, as many species are unable to produce a sufficient amount of AA by elongation of 18:2*n*–6, and even if they do, often numerous other unwanted C_{20} and C_{22} by-products may be formed (Sargent, 1995).

The specific inclusion of AA as a dietary EFA was targeted at the putative requirements of spiny lobster phyllosoma but may be incorporated at different rates to suit the requirements of a number of other crustacean and fish species. Recent research on larval penaeids (*Penaeus japonicus*, *P. semisulcatus* and *P. monodon*) (D'Souza & Loneragan, 1999) and finfish (*Paralichthys olivaceus* and *Sparus aurata*) (Estévez *et al.*, 1997; Koven *et al.*, 2001) suggests benefits such as a reduction in stress related mortality and improved pigmentation when AA was incorporated into dietary regimes.

AA is available in the marine environment in relatively small amounts however it contributes up to 8% to the total fatty acid profile of spiny lobster phyllosoma (Smith, 1999; Phleger *et al.*, 2001). While it is unusual for temperate and polar marine species to contain such high levels of AA (Sinclair *et al.*, 1986), this is not so for benthic species (Nichols *et al.*, 1998b; Nichols *et al.*, 1998c; Dunstan *et al.*, 1999). The inclusion of AA at levels approaching those observed in phyllosoma from a wild origin was obtained in *Artemia* enriched with OWL+OIL. However, the percentage inclusion of EPA may still be insufficient in *Artemia* enriched with this diet. It is the balance of *n*–3/*n*–6 fatty acids and in particular of EPA:AA ratio which has been signaled as important in this and other larval crustaceans (Sargent, 1995; D'Souza & Loneragan, 1999; Smith, 1999; Phleger *et al.*, 2001). In a number of other species, AA has been suggested as having a major role as a precursor of eicosanoids, the highly biologically active molecules linked to molting and stress response (Lytle *et al.*, 1990; Sargent, 1995). Eicosanoid production from AA (*n*–6 fatty acid) is modulated by EPA (*n*–3 fatty acid), and failure to supply these two EFA in the appropriate balance may result in adverse biochemical responses when fed to the predator organisms (Sargent, 1995).

It is the ability of 24-h enriched *Artemia*, in particular at Day 5, to resemble the EFA profile of their dietary source that shows promise for further manipulation to meet the needs of host crustacean and fish predators. Within a 24-h period, Day 5 *Artemia* fed the OWL+OIL

and A1 DHA Selco enrichments mirrored the percentage dietary inclusion levels of both AA and EPA. The incorporation of DHA into *Artemia*, at Day 5 was markedly less than its level in the enrichment, however greater losses occurred in younger (Day 2) *Artemia*. Estévez *et al.* (1998) found that, of the EFA, the incorporation rate of DHA was less during *Artemia* nauplii enrichment and the loss highest during starvation, a situation analogous to that found in Day 2 and 5 *Artemia* in this study. Lower DHA incorporation in juvenile *Artemia* compared to dietary inclusion levels may be associated with the inability of juveniles to preferentially assimilate DHA during 24-h enrichment. We consider that DHA did not undergo significant retroconversion to EPA, as often occurs in *Artemia* nauplii (Navarro *et al.*, 1999; Evjemo *et al.*, 2001), because EPA was incorporated at a rate equivalent to inclusion levels in both OWL+OIL and A1 DHA Selco diets, and appeared to increase independently of DHA.

The uptake and maintenance efficiency of various fatty acids in *Artemia* during 24-h enrichment to a large degree dictates the level that they should be included in the enrichments to attain a desired profile. Both AA and EPA were incorporated and effectively maintained in juvenile *Artemia* at levels similar to their inclusion in the enrichments. However, to achieve a fatty acid profile similar to the desired target species (spiny lobster phyllosoma), the percentage inclusion of EPA would need to be increased in the OWL+ OIL enrichment.

In conclusion, it appears that juvenile *Artemia* require the intake of suitably sized particulate matter to enable gut evacuation to occur. While gut content was not noticeably voided within 6 h of the cessation of enrichment, we also found that at 3 and 6 h the gut content did not make a significant contribution to the total lipid content or fatty acid profiles of juvenile *Artemia*. Therefore, any loss of gut content in juvenile *Artemia* as a result of predator feeding behavior is insignificant in terms of the lipid content or fatty acid composition shortly after the cessation of enrichment. Juvenile *Artemia*, a life stage seldom used in feeding regimes, demonstrated the ability to assume the EFA profile of their dietary source, in particular the AA and EPA profiles and to a lesser degree, DHA. This is a trait that has considerable potential for use in species that have diverse and perhaps non-traditional EFA requirements. Further research on optimizing *Artemia* EPA:AA ratios, decreasing the level of 18:2n-6 and increasing the n-3/n-6 ratio to reflect the composition of spiny lobster phyllosoma, is being conducted.

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Appendix Four

Comparative Biochemistry and Physiology Part B (2003) in preparation

Feeding and starvation effects on the lipid composition of early stage western rock lobster (*Panulirus cygnus*) phyllosoma

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Abstract

Total lipid, lipid class and fatty acid analysis were conducted on fed and starved stage I and II phyllosoma of the western rock lobster, *Panulirus cygnus*. In both stages the decrease in dry mass of starved larvae and increase in dry mass of *Artemia*-fed larvae was accompanied by a decrease and increase in lipid content, respectively. However, lipid only accounted for 6.7% and 35.0% of the decrease in dry mass in starved stage I and II larvae, respectively. Also, lipid only accounted for 6.2% and 19.2% of the increase in dry mass of fed stage I and II larvae, respectively. The major lipid classes in all phyllosoma samples were polar lipids (84.1-94.3%) followed by sterols (6.6-12.1%; mainly cholesterol). Gravimetrically, fed larvae increased predominantly in polar lipid, while in starved larvae polar lipid was the major lipid class catabolised, with sterol levels not changing. This lead to a significant decrease in relative abundance of polar lipid and increase in sterol abundance. Hydrocarbons, wax esters, diacylglyceryl ether, triacylglycerols and free fatty acids were all at minor levels (<5%). Fatty acid analysis showed six major groups present; 16:0, 18:1(n-9), 18:0, 20:4(n-6) (arachidonic acid), 20:5(n-3) (eicosapentaenoic acid) and 22:6(n-3) (docosahexaenoic acid). All of these major fatty acids increased gravimetrically in fed larvae and decreased in starved larvae. In starved larvae small decreases were seen in the relative contribution of eicosapentaenoic acid, docosahexaenoic acid, 16:1(n-7)c and 18:1(n-9)c, with arachidonic acid increasing. In fed larvae, most of the major fatty acids remained at a similar relative contribution, and larvae were able to accumulate arachidonic acid and eicosapentaenoic acid above the level (%) in *Artemia*, but not docosahexaenoic acid. The results are useful in the identification of nutrients required during development and as such with the design of diets used in phyllosoma culture.

Keywords: *Artemia*; Fatty acids; Lipid; *Panulirus cygnus*, Phyllosoma; Rock lobster; Starvation

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1. Introduction

With increased product demand and value, the aquaculture or enhancement of rock lobsters to increase production is currently receiving considerable interest due to fully exploited wild fisheries worldwide (Kittaka, 1994b; Phillips *et al.*, 2000). The western rock lobster, *Panulirus cygnus*, is found on the lower west coast of Australia and supports the world's largest rock lobster fishery (Phillips *et al.*, 2000). To ensure sustainability, a rock lobster aquaculture industry would need to be based upon culture of phyllosoma from hatching through their entire larval development (Kittaka & Booth, 1994; Crear *et al.*, 1998). A major hurdle in complete culture is the long and complex larval component of its life history. Although the larval life cycle has been completed in a number of species, only limited success has been achieved (Booth, 1995; Kittaka, 1997b), with many studies experiencing high mortality in early stages (Kittaka, 1988; Kittaka & Ikegami, 1988; Kittaka, 1997b).

Nutrition is regarded as key factor controlling survival and growth in crustacean larval culture (Mikami *et al.*, 1995). Nutritional studies on Palinuridae are very scarce, particularly on the larval stages that are difficult to rear (Kanazawa & Koshio, 1994). Phyllosoma have been reared on *Artemia* and mussel gonad with varying success (Kittaka, 1988; Kittaka & Ikegami, 1988; Illingworth *et al.*, 1997), however appropriate nutrition requires the identification of essential elements within the diet. Feed quality may be one of the reasons which cause low survival of the larvae, making it necessary to establish the nutritional requirements of the phyllosoma for successful culture (Kanazawa & Koshio, 1994). Lipid has been found to be of prime importance in crustacean larval stages (Sasaki *et al.*, 1986; Kattner *et al.*, 1994), and appears to be the main storage product in late stage phyllosoma, which is then used as an energy source during the non-feeding puerulus stage (Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a). As very little is known about the feeding of phyllosoma in the wild, there is a need for larval nutritional research (Phleger *et al.*, 2001).

Starvation experiments are one way to determine nutritional requirements of fish and crustacean larvae, as when an animal tends to retain a specific fatty acid (FA) level during starvation, this is interpreted as a high requirement of that specific FA (Olsen, 1998). Koven *et al.* (1989) found fish larvae (*Sparus aurata*) conserved important n-3 FA during starvation, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and stated this is reasonable as a biochemical strategy since these FA are more valuable as essential components of membranes than as an energy source. Biochemical studies during starvation and feeding of prawn larvae have also supplied valuable information on the best nutrient specification of food for the larvae (D'Souza, 1998). Changes in the biochemistry of phyllosoma larvae during starvation indicate their nutritional requirements and are an important basis for determining suitable diets for larval culture (Ritar *et al.*, 2003a).

The objective of this work was to examine which lipids (lipid class and fatty acids) were of greater nutritional importance for stage I and II *P. cygnus* phyllosoma by comparing patterns of conservation and loss in starved and fed larvae. The aim was to provide information which would be useful in defining suitable diets with profiles appropriate for phyllosoma culture.

2. Materials and methods

2.1. Broodstock

The study took place at the Western Australian Marine Research Laboratories. Broodstock animals were kept in the laboratory under 12L:12D light cycle and fed daily with live mussels (*Mytilus edulis*) and routinely with fish and abalone. Larvae used in the stage I experiment hatched from a lobster (128.3 mm carapace length) mated in the laboratory, with oviposition of eggs (24th May 2001) at 25°C and incubation in a tank at 22°C (32-34 ppt), with larvae hatching after 39 days incubation. Larvae used in the stage II experiment hatched from a lobster (105.0 mm carapace length) mated in the laboratory, with oviposition of eggs (29th July 2001) at 25°C and incubation in a tank at 19°C (32-34 ppt), with larvae hatching after 57 days incubation.

2.2. Experimental system

The system used for rearing the phyllosoma has been successfully used with southern rock lobster (*Jasus edwardsii*) phyllosoma (Ritar, 2001). Seawater used in the experiments was heated to 23°C, filtered to 1 μm and UV sterilized (UViVF-9, 30 W) before entering the circular 30 L plastic rearing tubs. Water entered the tubs through four equally-spaced nozzles (jets) positioned close to the bottom perimeter of the tub, with another two towards the bottom centre of the tub to provide a circular water flow, keeping phyllosoma moving in the water column. Each tub had a water flow of approximately 1 L/minute and the volume was maintained at a constant 10 L. Excess water exited the tubs through screens positioned on the side of the tubs. Phyllosoma were fed daily with *Artemia* at 3/mL. Every morning remaining *Artemia* were removed from the system by replacing the feeding filters (200 μm)

with cleaning filters (1500 μm) and allowing the *Artemia* to flush out of the tubs. The feeding filters were replaced and freshly enriched *Artemia* were then added to the tubs. Phyllosoma were transferred to clean tubs weekly. Phyllosoma that were starved during stages I and II received the same daily procedure except they were not fed. The number of larvae added to the tubs was estimated volumetrically and larvae were randomly stocked at approximately 1,500/tub.

2.3. Artemia

Phyllosoma were fed with *Artemia* (Great Salt lake) that had been on-grown for 4-5 days using Algamac 2000 (Biomarine, Aquafauna) and an *Isochrysis* marine algal concentrate (Reed Mariculture, USA). *Artemia* were enriched for 18 hours (two feedings, 16:00 and 02:00) with Algamac 2000 prior to feeding to the phyllosoma.

2.4. Sampling protocol

All phyllosoma samples for biochemical analysis were taken in triplicate, i.e. three tubs were used for each sample. For stage I analysis, phyllosoma were sampled at hatch (Day 0), fed and starved samples in the middle of stage I (Day 6), and a sample of fed larvae after molting at the beginning of stage II (Day 15). For stage II analysis, phyllosoma were sampled at hatch (Day 0), at the beginning of stage II (Day 15), fed and starved samples were taken in the middle of stage II (Day 20), and a sample of fed larvae after molting at the beginning of stage III (Day 26). Larval stages were measured from the anterior margin of the cephalic shield between the eyestalks, to the posterior of the abdomen and were staged according to Braine *et al.* (1979). Samples of enriched *Artemia* were also taken for analysis.

2.5. Lipid analyses

2.5.1. Lipid extraction

Phyllosoma and *Artemia* samples were filtered onto 47 mm Whatman glass fiber filters and washed with 0.5 M ammonium formate. Samples were stored at -80°C, freeze dried overnight, and weighed to determine dry mass (DM). Samples were quantitatively extracted overnight using a modified Bligh and Dyer (1959) one-phase methanol:chloroform:water extraction (2:1:0.8 v/v/v). The phases were separated the following day by the addition of chloroform and water to give a final solvent ratio of 1:1:0.9 v/v/v methanol:chloroform:water. The total solvent extract (TSE) was concentrated using a rotary evaporator at 40°C, blown down to dryness under nitrogen, and weighed to determine TSE. Samples were made up in a known volume of chloroform and stored at -20°C before analysis.

2.5.2. Lipid classes

To quantify individual lipid classes (LC), an aliquot of the TSE was analyzed using an Iatroscan MK V TH 10 thin layer chromatography-flame ionization detector (TLC-FID, Tokyo, Japan). Samples were applied in duplicate to silica gel SIII chromarods (5 μm particle size) using 1 μL disposable micropipettes. Chromarods were developed in a glass tank lined with pre-extracted filter paper. The primary solvent system used for lipid class separation was hexane:diethyl ether:acetic acid (60:17:0.1 v/v/v), a mobile phase resolving non-polar compounds such as wax esters (WE), triacylglycerols (TAG), free fatty acids (FFA) and sterols (ST). A second non-polar solvent system of hexane:diethyl ether (96:4 v/v) was also used to resolve hydrocarbons (HC) from WE, and TAG from diacylglycerol ether (DAGE). After development, the chromarods were oven dried and analyzed immediately. The FID was calibrated for each compound class (phosphatidylcholine, cholesterol, cholesteryl ester, oleic acid, squalene, TAG [derived from fish oil], WE [derived from fish oil] and DAGE [derived from shark oil], 0.1-10 μg range), and the peaks were quantified using DAPA software (Kalamunda, Western Australia).

Table 1.
The mass per individual (mg), lipid content (mg/g DM and mg/individual) and size of phyllosoma larvae of the western rock lobster, *Panulirus cygnus*, during feeding and starvation.

Sample	Mass/ind (mg)*	Lipid (mg/g DM)*	Lipid (mg/ind)*	Size (mm)#
Hatch (1)	0.08 ± 0.00^{a}	42.87 ± 1.18^{ab}	3.40 ± 0.07^{a}	1.76 ± 0.02^{a}
Stage I middle fed	0.09 ± 0.01^{ab}	45.52 ± 1.39^{ab}	4.17 ± 0.19^{ab}	
Stage I middle starved	0.06 ± 0.00^{f}	33.33 ± 4.32^{b}	1.87 ± 0.13^{e}	
Stage II beginning	0.10 ± 0.00^{bc}	47.99 ± 5.76^{a}	4.75 ± 0.52^{b}	
Hatch (2)	0.08 ± 0.01^{a}	53.49 ± 5.69^{ac}	4.32 ± 0.18^{ab}	1.81 ± 0.02^{b}
Stage II beginning	0.11 ± 0.00^{c}	68.89 ± 7.16^{c}	7.58 ± 0.88^{c}	
Stage II middle fed	0.15 ± 0.01^{d}	100.43 ± 6.84^{d}	14.78 ± 0.42^{d}	
Stage II middle starved	0.10 ± 0.00^{bc}	34.59 ± 4.51^{b}	3.38 ± 0.38^{a}	
Stage III beginning	0.22 ± 0.01^{e}	66.02 ± 5.88^{c}	14.63 ± 0.42^{d}	

Data within a column with different superscripts are significantly different (α=0.05).

* n=3; # n=20; DM, dry mass.

2.5.3. Fatty acids

An aliquot of the TSE was *trans*-met hylated to produce fatty acid methyl esters (FAME) using methanol:chloroform:conc. hydrochloric acid (10:1:1 v/v/v) at 80°C for 2 hours. The FAME produced were extracted into hexane:chloroform (4:1 v/v, 3 x 1.5 mL) and treated with N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA, 100 µL, 70°C, overnight) to convert ST and alcohols to their corresponding TMSi (trimethylsilyl) ethers. Samples were blown down under nitrogen and an internal standard (C19 and C23, 40mg/g) was added.

Gas chromatographic (GC) analyses were performed with a Hewlett Packard 5890A GC (Avondale, PA) equipped with a HP-5 cross linked methyl silicone fused silica capillary column (50 m x 0.32 mm i.d.), a FID, a split/splitless injector, and a HP 7673A auto sampler. Helium was used as the carrier gas. Samples were injected in splitless mode at an oven temperature of 50°C. After 1 minute the oven temperature was raised to 150°C at 30°C/minute, then to 250°C at 2°C/minute, and finally to 300°C at 5°C/minute. Peaks were quantified with Waters Millennium software (Milford, MA, USA). Individual components were identified using mass spectral data and by comparing the retention time data with those obtained for authentic and laboratory standards. GC results are subject to an error of ±5% of individual component area. GC-mass spectrometric (GC-MS) analyses were performed on a Finnigan Thermoquest GCQ GC-mass spectrometer (Austin, TX, USA) fitted with an on-column injector. The GC was fitted with a capillary column similar to that described above.

2.6. Statistical analyses

Results were analyzed using one-way ANOVA with Tukey's test used for multiple comparisons. Percentage data was arcsine$\sqrt{}$ transformed and gravimetric data $\sqrt{}$ transformed to make the data normal and homogenous. When two samples were compared (hatch sizes) a t-test was used. Statistical analyses were performed using Statistica software (StatSoft Inc., USA, version 6). Data is presented as mean±SD, and results were considered significantly different at $P \leq 0.05$.

3. Results

For stage I samples comparisons between changes in fed and starved larvae will be compared to newly hatched larvae (from first hatch), and for stage II samples to newly molted stage II larvae (from the second hatch).

Table 2.
Percentage lipid class composition of western rock lobster (*Panulirus cygnus*) phyllosoma and *Artemia*.

Sample	Hydrocarbons	Wax esters	DAGE	TAG	Free fatty	Sterols	Polar lipids
Stage I							
hatch 1	2.5 ± 1.23^{abc}	1.3 ± 0.53^{ab}	0.1 ± 0.0	0.1 ± 0.04^{ab}	0.5 ± 0.05^{ab}	7.7 ± 0.57^{ab}	87.8 ± 1 36^{ad}
middle fed	1.6 ± 0.24^{acd}	0 3 ± 0.14^{bcd}	0.1 ± 0.1	0.1 ± 0.02^{ab}	0.2 ± 0.07^{a}	8.2 ± 0.37^{ab}	89.6 ± 0 38^{ab}
middle starved	4 6 ± 1.38^{b}	2.3 ± 0.65^{a}	0.1 ± 0.1	0.2 ± 0.11^{b}	1.1 ± 0.58^{b}	9.8 ± 0.71^{ad}	82 0 ± 2.66^{e}
Stage II - beginning	2.9 ± 0.62^{ab}	1.0 ± 0.29^{ad}	0.0 ± 0.0	0.2 ± 0.13^{ab}	0.4 ± 0.37^{a}	7.3 ± 1.37^{bc}	88.3 ± 0.59^{abd}
Stage I – hatch 2	0.7 ± 0.19^{cd}	0.1 ± 0.05^{c}	0.0 ± 0.0	0.1 ± 0.02^{ab}	0.1 ± 0.04^{a}	7.2 ± 0.55^{bc}	91.8 ± 0.32^{bc}
Stage II							
beginning	1.1 ± 0.79^{acd}	0.6 ± 0.56^{bcd}	0.1 ± 0.0	0.1 ± 0.02^{ab}	0.3 ± 0.15^{a}	6.6 ± 0.86^{bc}	91.4 ± 2.26^{abc}
middle fed	0.4 ± 0.23^{d}	0.1 ± 0.04^{c}	0.0 ± 0.0	0.0 ± 0.01^{a}	0.1 ± 0.02^{a}	5.1 ± 0.96^{c}	94.3 ± 0.72^{c}
middle starved	2.6 ± 1.24^{abc}	0.6 ± 0.39^{bcd}	0.1 ± 0.0	0.1 ± 0.02^{ab}	0.5 ± 0 14^{ab}	12.1 ± 0.58^{d}	84.1 ± 1.32^{de}
Stage III - beginning	0.7 ± 0.35^{cd}	0.2 ± 0.06^{cd}	0.1 ± 0.0	0.1 ± 0.01^{ab}	0.1 ± 0.05^{a}	7.1 ± 0.91^{bc}	91.8 ± 1.20^{ab}
Artemia	0.3 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	31.0 ± 2.7	3.1 ± 0.2	3.3 ± 0.3	62.0 ± 2.6

Data within a column with different superscripts are significantly different (□=0.05); TAG, triacylglycerol; DAGE, diacylglyceryl ether

3.1. Phyllosoma and lipid amounts

The DM increased in stage I and II fed larvae (15.7 and 34.1%, respectively) and conversely decreased in starved stage I and II larvae (28.8 and 10.9%, respectively) (Table 1). Larvae also showed increases in dry mass (DM) when they molted to each stage, with the increase from stage II to III much greater than from stage I to II (Table 1). As with DM, the lipid content (mg/g DM and μg/phyllosoma) of stage I and II larvae increased in fed samples and decreased in starved samples (Table 1). In stage I larvae, the increase in lipid content of fed larvae accounted for 6.2% of the increase in DM, while the lipid decrease in starved larvae accounted for 6.7% of the decrease in DM. In stage II larvae, lipid accounted for a much greater percentage of the increase in DM of fed larvae, 19.2%, and also accounted for a much larger portion of the decrease in starved stage II larvae, 35.0%. Although newly molted stage III did have a similar amount of lipid/phyllosoma compared to fed stage II larvae, total lipid (mg/g DM) decreased significantly after the moult (Table 1).

Larvae from the two hatches did not significantly differ in DM or lipid amounts (mg/g DM and μg/phyllosoma), however the lipid amounts were slightly elevated in larvae from the second hatch. These larvae were also significantly larger at hatch (Table 1). Stage II larvae from the two hatches however differed significantly, with newly molted stage II from the first hatch having significantly less lipid (Table 1).

3.2. Lipid class

In all phyllosoma samples, PL was the major LC (82.0-94.3%) (Table 2). PL was also the major LC used during starvation and was significantly reduced in starved stage I and II larvae (Table 2, Figure 1). The amount of PL in starved stage I larvae (27.2 mg/g DM) was 27.6% less than in newly hatched larvae (37.6 mg/g DM), and increased by 8.4% in fed phyllosoma (40.8 mg/g DM). For stage II larvae, the amount of PL in starved larvae (29.1 mg/g DM) was 53.9% less than in newly molted stage II larvae (63.0 mg/g DM), and increased by 50.2% in fed stage II larvae (94.7 mg/g DM). In both stages, the increase in PL (mg/g) almost solely accounted for the increase in lipid (mg/g). ST were the next most abundant lipid class (largely cholesterol) comprising between 5.1-12.1% of the total lipid (Table 2). In relative proportions, ST levels (%) increased significantly in starved larvae, with no significant decrease in amount (mg/g DM) (Table 2, Figure 1). This was followed by HC (0.7-4.6%), which also showed an increase in relative abundance (%) and amount (mg/g DM) in starved larvae. WE were the next most abundant LC (0.1-2.3%), followed by FFA (0.1-1.1%), DAGE and TAG (≤0.2%) (Table 2).

As with lipid amounts, PL levels differed in larvae from the different hatches, larvae from the second hatch (49.1 mg/g DM) having significantly increased levels compared to

larvae from the first hatch (37.6 mg/g DM) (Table 2, Figure 1). The gravimetric amount of PL (mg/g DM) was still significantly low er in newly molted stage II larvae from the first hatch (42.4 mg/g DM) compared to larvae from the second hatch (63.0 mg/g DM) (Table 2, Figure 1).

Enriched *Artemia* contained high levels of PL (62.0%; 83.9 mg/g DM), although unlike the phyllosoma samples, also had high levels of TAG (31.0%; 41.8 mg/g DM). This was followed by ST (3.3%; 4.5 mg/g DM), and FFA (3.1%; 4.2 mg/g DM), with HC, DAGE and WE minor components (≤0.3%; <0.5 mg/g DM) (Table 2, Figure 1).

3.3. Fatty acids

Of the 55 fatty acids (FA) identified, 18 individual FA had some concentrations ≥1% in the phyllosoma samples. Both hatches showed a similar relative contribution for the major FA present (≥5%) (Tables 3 and 4). The six most abundant FA were 20:5n-3 (EPA, 13.7-21.3%), 16:0 (palmitic acid, 10.7-15.2%), 18:1n-9 (oleic acid, 10.6-14.6%), 22:6n-3 (DHA, 7.0-13.0%), 20:4n-6 (arachidonic acid, AA, 5.8-12.4%) and 18:0 (stearic acid, 8.5-13.9%) (Tables 3 and 4).

The major FA in fed stage I larvae showed similar relative contribution to newly hatched larvae, except there was a significant increase in EPA and decrease in 16:0 (Table 3). Similarly, fed stage II larvae showed a similar relative contribution in the major FA compared to newly molted stage II larvae, although 16:0 was significantly elevated (Table 4). Gravimetrically, total FA (mg/g DM) increased significantly in fed stage I and II larvae as did the major FA (Tables 3 and 4, Figure 2). The sum of SFA and MFA in fed stage I and II larvae did not change. The sum of PUFA did not significantly change in fed stage I and II larvae, however there was a small increase (Table 3 and 4). Both stage I and II fed larvae showed an increase in n-3/n-6 ratio (significant in stage I) (Tables 3 and 4). The EPA/AA ratio showed a significant increase in both fed stage I and II larvae, and a reduction in starved larvae (not significant) (Tables 3 and 4). The DHA/EPA ratio did not change in fed or starved stage I or II larvae.

Starved stage I and II larvae showed a gravimetric decrease in total FA, although this was only significant in stage II larvae (Tables 3 and 4). Individually, most of the major FA decreased in starved stage I larvae, although only DHA significantly, and 18:0 actually increased (Figure 2). In starved stage II larvae, all the major FA significantly decreased gravimetrically. On a percentage basis, AA increased in starved stage I and II larvae (significant in stage II) (Table 3 and 4). EPA and DHA decreased in starved stage I and II larvae, although this was not significant (Table 3 and 4). Starved stage I larvae showed no significant difference in the sum of MFA or PUFA compared to newly hatched larvae, however SFA significantly increased (Table 3). Starved stage II larvae did not show a difference in the sum of SFA, however PUFA significantly increased and MFA significantly decreased (Table 4). In stage I and II starved larvae, there was no change in the sum of n-6. The sum n-3 decreased significantly in starved stage I larvae with no change in starved stage II larvae (Tables 3 and 4).

The total FA content significantly decreased in newly molted stage III larvae compared to fed stage II larvae. However, newly molted stage II larvae actually contained a higher content than fed stage I larvae (Table 3 and 4).

Gravimetrically, newly hatched larvae from the second hatch were significantly elevated in total FA and all the major FA (Tables 3 and 4, Figure 2). Newly molted stage II larvae from the second hatch also had significantly higher total FA levels and significantly higher major FA levels than newly molted stage II larvae from the first hatch (Tables 3 and 4, Figure 2).

The percentage FA composition of the *Artemia* was dominated by 16:0 (19.7%), followed by DHA (14.3%), 18:1n-9 (12.3%), EPA (10.7%), 18:1n-7 (9.8%), and 16:1n-7c (6.2%). Other FA were present at ≤6%, with AA at 2.3% (Table 3). Phyllosoma were able

Table 3.
Percentage fatty acid (FA) composition and total FA levels (mg/g DM) at hatch, after feeding, starvation and moulting of western rock lobster (*Panulirus cygnus*) phyllosoma from Hatch 1 and *Artemia*.

	Stage I			Stage II	*Artemia*
	hatch	fed	starved	beginning	
14:0	0.9 ± 0.13^{ab}	0.6 ± 0.04^{bd}	1.0 ± 0.31^{ab}	1.2 ± 0.05^{ac}	5.1 ± 0.0
16:1n-7c	2.8 ± 0.19^{ac}	1.6 ± 0.07^{b}	2.2 ± 0.39^{fg}	2.6 ± 0.10^{af}	6.2 ± 0.1
16:0	12.2 ± 0.80^{a}	10.7 ± 0.35^{b}	12.1 ± 0.15^{a}	13.9 ± 0.50^{c}	19.7 ± 0.2
17:0	0.8 ± 0.12^{a}	0.9 ± 0.03^{bd}	1.1 ± 0.04^{c}	1.1 ± 0.02^{c}	0.7 ± 0.0
18:2n-6	1.1 ± 0.15^{a}	1.4 ± 0.04^{ac}	1.3 ± 0.21^{ac}	1.9 ± 0.05^{b}	1.7 ± 0.0
18:1n-9c	11.2 ± 0.64^{ad}	11.2 ± 0.32^{ad}	10.7 ± 0.98^{ad}	14.6 ± 0.34^{b}	12.3 ± 0.1
18:1n-7c	3.4 ± 0.39^{a}	5.3 ± 0.14^{b}	3.7 ± 0.23^{a}	9.1 ± 0.24^{c}	9.8 ± 0.1
18:0	8.5 ± 0.22^{a}	9.9 ± 0.21^{b}	12.3 ± 0.49^{cd}	11.9 ± 0.65^{c}	5.3 ± 0.1
18:0 FAde	2.8 ± 0.19^{a}	2.0 ± 0.15^{b}	3.7 ± 0.20^{d}	1.6 ± 0.29^{c}	0.0 ± 0.0
20:4n-6 AA	11.2 ± 0.95^{ae}	10.8 ± 0.91^{abe}	12.4 ± 0.82^{e}	8.7 ± 0.15^{bc}	2.3 ± 0.0
20:5n-3 EPA	16.7 ± 1.07^{a}	21.3 ± 0.99^{b}	15.6 ± 0.69^{ac}	14.9 ± 0.01^{ac}	10.7 ± 0.1
20:2n-6	0.8 ± 0.11^{a}	0.7 ± 0.02^{a}	1.2 ± 0.04^{c}	0.5 ± 0.04^{b}	0.1 ± 0.0
20:1(n-9/11)c	2.1 ± 0.29^{af}	1.3 ± 0.05^{b}	1.8 ± 0.06^{f}	1.1 ± 0.03^{bc}	0.3 ± 0.0
20:00	0.5 ± 0.11^{a}	0.8 ± 0.02^{bc}	1.4 ± 0.09^{e}	0.9 ± 0.03^{b}	0.2 ± 0.0
22:5n-6	0.4 ± 0.08^{a}	0.9 ± 0.05^{b}	0.2 ± 0.03^{e}	1.4 ± 0.07^{cf}	4.7 ± 0.2
22:6n-3 DHA	11.5 ± 0.84^{ac}	13.0 ± 0.44^{a}	10.3 ± 0.52^{ce}	7.1 ± 0.17^{bd}	14.3 ± 0.7
22:5n-3	0.7 ± 0.13^{a}	0.3 ± 0.02^{b}	0.3 ± 0.06^{b}	0.2 ± 0.01^{c}	0.2 ± 0.0
20:0	0.5 ± 0.09^{a}	0.9 ± 0.03^{b}	1.3 ± 0.05^{c}	1.2 ± 0.02^{cd}	0.7 ± 0.0
Other	8.5 ± 1.0	6.2 ± 0.6	8.0 ± 0.5	6.6 ± 0.6	6.1 ± 0.3
Sum SFAs	24.8 ± 0.96^{a}	24.8 ± 0.69^{a}	30.5 ± 0.32^{cd}	31.7 ± 0.89^{bc}	34.0 ± 0.3
Sum MFAs	21.7 ± 0.66^{ad}	20.8 ± 0.69^{ad}	19.7 ± 1.59^{d}	28.9 ± 0.27^{b}	30.3 ± 0.3
Sum PUFA	45.3 ± 1.90^{ac}	50.4 ± 1.85^{a}	43.6 ± 1.67^{c}	36.5 ± 0.38^{bde}	48.1 ± 0.5
Sum n-3	29.3 ± 1.80^{a}	35.2 ± 1.16^{b}	26.8 ± 1.26^{ac}	23.0 ± 0.27^{cd}	38.4 ± 0.4
Sum n-6	14.9 ± 0.62^{ab}	14.5 ± 0.38^{ab}	15.8 ± 0.46^{a}	13.1 ± 0.06^{bc}	9.3 ± 0.1
Ratio (n-3)/(n-6)	2.0 ± 0.20^{ab}	2.4 ± 0.31^{c}	1.7 ± 0.08^{a}	1.8 ± 0.03^{a}	4.1 ± 0.0
Ratio EPA/AA	1.5 ± 0.13^{acd}	2.0 ± 0.33^{b}	1.3 ± 0.06^{d}	1.7 ± 0.03^{ab}	4.6 ± 0.0
Ratio DHA/EPA	0.7 ± 0.04^{a}	0.6 ± 0.04^{b}	0.7 ± 0.02^{ab}	0.5 ± 0.01^{c}	1.3 ± 0.1
Total FA (mg/g DM)	14.8 ± 0.43^{a}	21.6 ± 2.51^{b}	13.1 ± 1.63^{a}	23.6 ± 1.79^{b}	78.1 ± 2.3

Data within a row with different superscripts are significantly different (=0.05).

AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; NMI, non-methylene interrupted; Fade, fatty aldehyde; DM, dry mass; Other < 1%: 12:0, i14:0, 14:1, C14PUFA, i15:0, a15:0, 15:1, 15:0, i16:0, C16PUFA, 16:1n-9c, 16:2, 16:1n-5c, 16:0 Fade, br17:1, i17:0, a17:0, '17:1, 18:3n-6, i18:0, 18:4n-3, 18:3n-3, 18:1n-5c, C19PUFA, i19:0, 19:1, 20:2, 20:3n-6, 20:2NMI, 20:4n-3 20:1n-7c, C20Fade, C21PUFA, 21:0, 22:4n-6, 22:1, C28PUFA

to accumulate AA and EPA at % levels above that in *Artemia*, but not DHA, which was always lower in phyllosoma.

4. Discussion

4.1. Changes in phyllosoma and lipid amounts

In both larval stages, there were increases in the DM and in the amount of lipid in fed larvae, with decreases in starved larvae. These increases and decreases were more significant in stage II larvae (Table 1). Lipids have been found to be used during starvation in other crustaceans (Virtue *et al.*, 1993), and previous research has found lipid important in all

Table 4.
Percentage fatty acid (FA) composition and total FA levels (mg/g DM) at hatch, after feeding, starvation and moulting (mean ± SD; n = 3), of western rock lobster (*Panulirus cygnus*) phyllosoma from Hatch 2.

	Stage I	Stage II			Stage III
	hatch	beginning	fed	starved	beginning
14:0	0.4 ± 0.03^{d}	1.2 ± 0.16^{ac}	1.4 ± 0.06^{c}	1.1 ± 0.16^{ac}	1.2 ± 0.05^{ac}
16:1n-7c	2.7 ± 0.02^{a}	3.2 ± 0.04^{cd}	3.7 ± 0.07^{de}	1.8 ± 0.11^{bg}	3.8 ± 0.16^{e}
16:0	13.7 ± 0.04^{c}	14.0 ± 0.24^{c}	15.2 ± 0.26^{d}	12.6 ± 0.13^{a}	14.6 ± 0.33^{cd}
17:0	1.2 ± 0.01^{c}	0.9 ± 0.01^{bd}	0.9 ± 0.02^{ab}	1.0 ± 0.03^{bc}	1.1 ± 0.03^{cd}
18:2n-6	1.2 ± 0.01^{ac}	1.9 ± 0.02^{b}	1.9 ± 0.01^{b}	1.5 ± 0.11^{c}	1.9 ± 0.04^{b}
18:1n-9c	12.4 ± 0.03^{ac}	13.8 ± 0.12^{bc}	14.5 ± 0.11^{b}	10.6 ± 0.23^{d}	13.6 ± 0.15^{bc}
18:1n-7c	4.5 ± 0.01^{d}	10.4 ± 0.49^{e}	12.0 ± 0.10^{f}	8.2 ± 0.08^{g}	12.3 ± 0.22^{f}
18:0	9.8 ± 0.06^{ab}	11.8 ± 0.11^{c}	11.7 ± 0.14^{c}	13.9 ± 0.12^{d}	12.8 ± 0.18^{cd}
18:0 Fade	3.8 ± 0.04^{d}	1.5 ± 0.14^{c}	0.8 ± 0.02^{e}	2.3 ± 0.19^{ab}	1.0 ± 0.04^{e}
20:4n-6 AA	9.9 ± 0.02^{ab}	7.4 ± 0.26^{cd}	5.8 ± 0.03^{d}	9.8 ± 0.17^{ab}	6.0 ± 0.14^{d}
20:5n-3 EPA	13.7 ± 0.05^{c}	14.9 ± 0.12^{ac}	14.2 ± 0.16^{ac}	16.4 ± 0.14^{ac}	14.6 ± 0.23^{ac}
20:2n-6	1.3 ± 0.01^{c}	0.5 ± 0.03^{b}	0.3 ± 0.02^{d}	0.7 ± 0.05^{a}	0.3 ± 0.01^{d}
20:1(n-9/11)c	2.3 ± 0.03^{a}	1.0 ± 0.04^{cd}	0.8 ± 0.02^{de}	1.0 ± 0.02^{cd}	0.8 ± 0.02^{e}
20:0	0.7 ± 0.01^{bc}	0.8 ± 0.05^{bc}	0.6 ± 0.01^{ad}	1.1 ± 0.02^{f}	0.7 ± 0.03^{cd}
22:5n-6	0.4 ± 0.01^{a}	1.6 ± 0.09^{e}	1.9 ± 0.07^{d}	1.2 ± 0.02^{f}	1.6 ± 0.08^{e}
22:6n-3 DHA	10.5 ± 0.05^{c}	7.9 ± 0.16^{bd}	7.8 ± 0.30^{bd}	8.6 ± 0.09^{be}	7.0 ± 0.33^{d}
22:5n-3	1.1 ± 0.01^{d}	0.2 ± 0.02^{ce}	0.2 ± 0.01^{ce}	0.1 ± 0.01^{f}	0.1 ± 0.00^{ef}
22:0	0.9 ± 0.02^{b}	1.2 ± 0.13^{cd}	1.0 ± 0.04^{bd}	1.8 ± 0.00^{e}	1.2 ± 0.06^{cd}
Other	10.1 ± 0.0	6.4 ± 0.1	5.8 ± 0.1	7.3 ± 0.7	6.1 ± 0.2
Sum SFAs	28.2 ± 0.10^{d}	31.6 ± 0.32^{bc}	32.5 ± 0.42^{bc}	33.0 ± 0.58^{b}	33.2 ± 0.41^{b}
Sum MFAs	24.7 ± 0.07^{e}	29.9 ± 0.49^{bc}	32.5 ± 0.22^{c}	22.8 ± 0.26^{ae}	31.9 ± 0.49^{c}
Sum PUFA	41.5 ± 0.22^{bc}	26.0 ± 0.17^{de}	33.6 ± 0.65^{e}	40.2 ± 0.18^{bcd}	32.9 ± 0.79^{e}
Sum n-3	25.9 ± 0.10^{acd}	23.7 ± 0.19^{cd}	22.9 ± 0.48^{cd}	25.8 ± 0.20^{acd}	22.3 ± 0.60^{d}
Sum n-6	14.3 ± 0.04^{ab}	11.9 ± 0.15^{cd}	10.5 ± 0.13^{de}	13.6 ± 0.06^{bc}	10.2 ± 0.20^{e}
Ratio (n-3)/(n-6)	1.8 ± 0.00^{a}	2.0 ± 0.04^{ab}	2.2 ± 0.02^{bc}	1.9 ± 0.01^{ab}	2.2 ± 0.02^{bc}
Ratio EPA/AA	1.4 ± 0.00^{cd}	2.0 ± 0.09^{b}	2.4 ± 0.02^{e}	1.7 ± 0.02^{abc}	2.4 ± 0.02^{e}
Ratio DHA/EPA	0.8 ± 0.00^{e}	0.5 ± 0.01^{cd}	0.6 ± 0.00^{d}	0.5 ± 0.00^{cd}	0.5 ± 0.02^{c}
Total FA (mg/g DM)	27.4 ± 3.22^{bc}	33.1 ± 0.91^{c}	57.8 ± 7.54^{d}	14.0 ± 0.74^{a}	33.7 ± 2.94^{c}

larval stages of spiny lobsters (Jeffs *et al.*, 1999; Phleger *et al.*, 2001). However, lipid only accounted for 6.7% of the decrease in DM in starved stage I larvae, while this increased to 35.0% for stage II larvae. Also, lip id only accounted for 6.2% and 19.2% of the increase in DM of fed stage I and II larvae, respectively. This indicates that lipid was not the major nutrient catabolised during starvation or accumulated by the feeding phyllosoma. Smith *et al.* (2003c) suggested that protein, and to a lesser extent carbohydrate, would have been catabolised during starvation of stage I *J. edwardsii* phyllosoma in which lipid only accounted for 17.8% in the loss of DM.

Larvae molting to successive stages also showed an increase in DM, with stage II larvae molting to stage III also showing a reduction in the lipid content (Table 1), possibly used during the moult. Smith *et al.* (2003c) speculated the increase in lipid accumulation seen in fed *J. edwardsii* phyllosoma might be used during the subsequent moult. American lobster (*Homarus americanus*) larvae also showed a reduction in lipid during molting (Sasaki, 1984). A decrease was not seen in newly molted stage II *P. cygnus* larvae compared to fed stage I, the lipid level was actually higher in the newly molted stage II larvae (Table 1). This may be attributable to a large variation in the molting period of the larvae. Larvae from

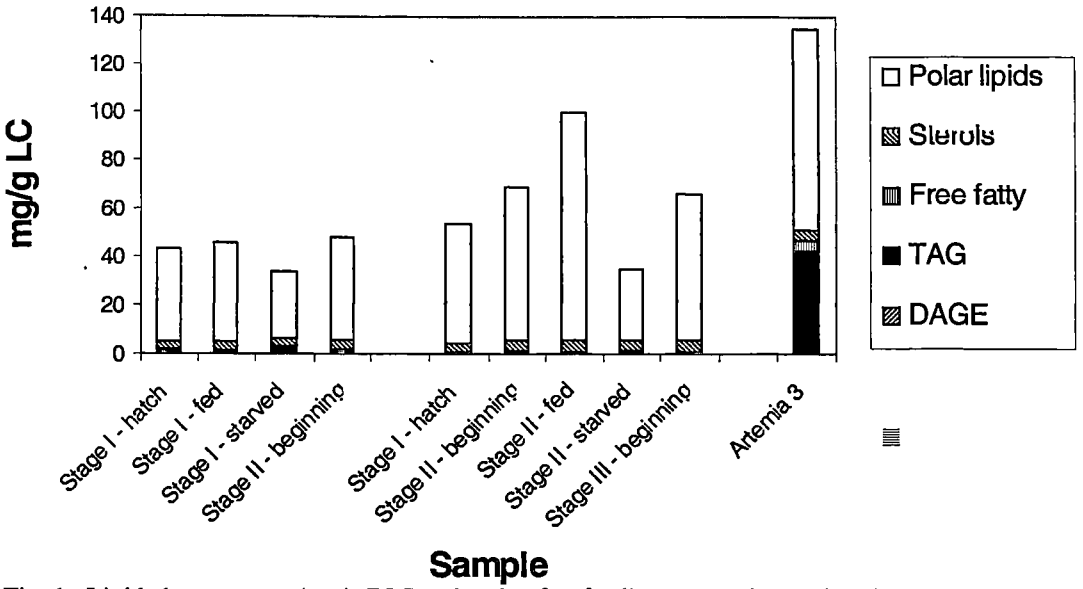

Fig. 1. Lipid class content (mg/g DM) at hatch, after feeding, starvation and molting (n = 3), of western rock lobster (*Panulirus cygnus*) phyllosoma and in *Artemia*. LC, lipid class; TAG, triacylglycerol; DAGE, diacylglyceryl ether; DM, dry mass

the first hatch took three days to all moult to stage II before a sample was taken. Therefore, many of the sampled larvae may have started to accumulate lipid.

4.2. Lipid class

The absence or very low levels of TAG in phyllosoma samples has been a common feature found in studies so far (Phleger *et al.*, 2001; Nelson *et al.*, 2003a; Nelson *et al.*, 2003b; Ritar *et al.*, 2003a), including this study (Table 2). TAG molecule is generally the most common lipid source in animals, used as a short-term energy reserve and is generally catabolised primarily during starvation (Koven *et al.*, 1989; Olsen, 1998; Phleger *et al.*, 2001). This is in contrast to PL which plays an important structural role and is usually preferentially conserved (Koven *et al.*, 1989). However, PL was the major LC in *P. cygnus* larvae comprising 82.0-94.3% of the total lipid from all samples (Table 2), as found with *J. edwardsii* phyllosoma (Phleger *et al.*, 2001; Nelson *et al.*, 2003a; Nelson *et al.*, 2003b; Ritar *et al.*, 2003a). In this study the increase found in lipid (mg/g DM) in both stages of fed *P. cygnus* larvae corresponded almost completely to the increase in PL (mg/g DM). The use of PL as the dominant storage medium in the puerulus stage is unlike many other marine taxa, including Crustacea, which tend to use TAG and WE (Sasaki, 1984; Jeffs *et al.*, 2001a). Jeffs *et al.* (2001a) found PL reserves were primarily utilised during the puerulus stage, and suggested the use of PL as a storage medium in the puerulus may be related to its characteristic transparency, an important feature of this nektonic stage that is highly vulnerable to pelagic visual predators. Jeffs *et al.* (2001a) suggested pueruli avoid using neutral lipid as a storage formate because of its opaque nature. The prevalence of PL as the major LC in phyllosoma larvae may be due to their transparency, which like the puerulus, could provide protection during their long larval phase in the water column.

PL was also the major LC used during starvation and was significantly reduced in starved larvae from both stages (Table 2, Figure 1). Lipid was found to be the primary energy source used during the non-feeding puerulus stage and PL was the major LC used (Jeffs *et al.*, 1999; Jeffs *et al.*, 2001a). Virtue *et al.* (1993) found the lipid content of the digestive gland of Antarctic krill (*Euphausia superba*) decreased significantly during starvation and that PL was the major LC used during starvation. This is unlike *H. americanus* larvae in which TAG showed a rapid depletion during starvation followed by PL (Sasaki, 1984). PL

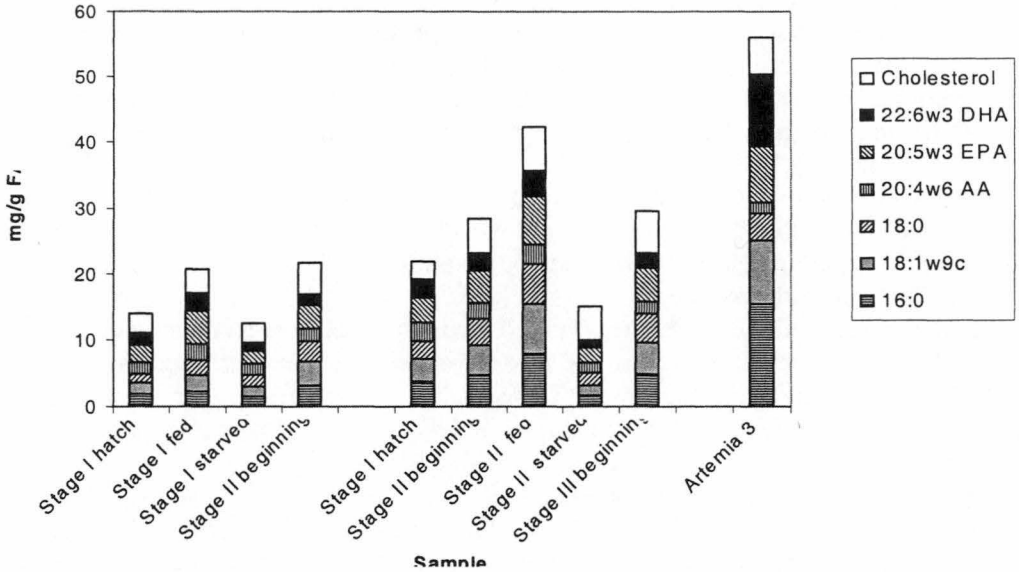

Fig. 2. The major fatty acid content (mg/g DM) at hatch, after feeding, starvation and moulting (n = 3), of western rock lobster (*Panulirus cygnus*) phyllosoma and in *Artemia*. FA, fatty acid; DM, dry mass

was also the only LC to be depleted when stage II larvae moulted to stage III, with a significant gravimetric reduction compared to fed stage II larvae (Figure 1).

ST (mainly cholesterol) was the next most abundant LC in the phyllosoma samples (Table 2). PL, together with cholesterol and sphingolipids, are omnipresent components of cell membranes and are therefore both structurally and functionally important (Olsen, 1998). Unlike PL, relative amounts of ST increased in starved larvae, with no significant decrease in gravimetric amount (mg/g DW). This retention may indicate the importance of ST, as ST are used not only in membranes but also in the transport of lipids (Ackman, 1998). Other studies with crustacean larvae have also found the ST content did not change during starvation (Sasaki, 1984; Virtue *et al.*, 1993).

As with other phyllosoma studies (Phleger *et al.*, 2001; Nelson *et al.*, 2003a; Nelson *et al.*, 2003b; Ritar *et al.*, 2003a), HC, WE, FFA, DAGE and TAG were all present at minor levels in the present study for all samples (Table 2). FFA did increase slightly in starved larvae (Table 2), likely due to the breakdown of the PL and release of FFA. Virtue *et al.* (1993) also found FFA increased with starvation and speculated this to be the result of the PL being metabolized as a source of metabolic energy.

Phyllosoma were able to maintain a higher percentage of PL and ST than found in the their diet (Table 2). *Artemia* contained lower levels of PL (62.0%) and ST (3.3%), with higher levels of TAG (31.0%) and FFA (3.1%) than phyllosoma (Table 2). It appears PL and ST are important nutrients to the phyllosoma, hence their accumulation at levels (%) above that found in their food. However, TAG is almost absent (<0.2%) in the phyllosoma samples (Table 2).

4.3. Fatty acids

The major FA and their profiles in newly hatched *P. cygnus* larvae (Tables 3 and 4) are similar to those reported for newly hatched *J. edwardsii* phyllosoma (14.2-14.6% 16:0, 12.6-13.3% 18:1n-9c, 6.9-7.1% 18:0, 11.6-11.9% AA, 14.8-15.3% EPA, and 7.6-8.1% DHA) (Phleger *et al.*, 2001). Fed *P. cygnus* larvae also showed a similar relative contribution from the major FA, even though all the major FA significantly increased gravimetrically. Although the major FA increased gravimetrically in fed larvae, the relative levels were much different than found in their diet. The FA distribution of animals is believed to be primarily determined by the composition of their dietary FA (Napolitano, 1998; Olsen, 1998). How-

ever, in the present study 16:0 and DHA were at lower relative levels than found in the *Artemia*, with 18:0, AA and EPA at levels above that found in the *Artemia*, possibly indicating a preferential accumulation of these FA. DHA showed a continual reduction in relative contribution as the phyllosoma developed (fed and moulting samples). This may suggest that the DHA was not in a form that the phyllosoma could absorb and use, or was not at high enough levels. AA also showed a fall throughout development, however the level of AA in the *Artemia* was always much lower than found in the phyllosoma. The phyllosoma lipid was around 90% PL, and comparatively the PL in *Artemia* was quite low (60%). TAG makes up a large portion in *Artemia*, and it's possible phyllosoma cannot absorb or accumulate the TAG to gain nutrients.

The increase in the EPA/AA ratio in fed larvae suggests a higher accumulation of EPA in fed larvae, however starved larvae did give a reduced ratio (not significant) suggesting more AA was conserved during starvation (Table 3 and 4). The DHA/EPA ratio did not change in fed or starved stage I or II larvae (Tables 3 and 4). Equal ratios in starved larvae compared to initial samples suggests both were used/retained at a similar rate.

In order to formulate a diet that meets EFA requirements of a given species, it is necessary to know the dominant EFA series for this species (Corraze, 2001). Experiments have found that during starvation larvae conserve important FA, with starvation suggested as one way to determine nutritional requirements of larvae (Koven *et al.*, 1989; Olsen, 1998). Starved *P. cygnus* larvae showed a gravimetric decrease in total FA, however a number of individual FA showed no change or assumed a greater ranking in the FA profile, possibly signifying a higher level of importance. The FA changes were however dependent upon the stage tested. The relative contribution of AA increased in starved stage I and II larvae, although the change in starved stage I larvae was not significant. The conservation or increase in relative contribution of may indicate high importance for the larvae. EPA and DHA showed increases in stage II larvae, however in starved stage I larvae there was a decrease (not significant).

Starved stage I larvae showed a significant increase in SFA, with no change in MFA or PUFA. However in starved stage II larvae MFA significantly decreased, whereas PUFA significantly increased, possibly indicating a preferential use of MFA during starvation and retention of PUFA. Kattner *et al.* (1994) found the FA profile of larval caridean shrimp also showed major differences dependant upon stage and larval sample. The variation in results in the present study suggest a response which is dependent upon the stage of the larvae.

4.4. Variation between hatches

Newly hatched larvae from the second hatch had elevated levels of lipid (PL and total FA) compared to larvae from the first hatch, with larvae from the second hatch also significantly larger (Table 1, 2, 3 and 4). This may be attributable to different temperatures during egg incubation. Both females extruded eggs at 25°C, however eggs from the first hatch were incubated in water at 22°C, while those from the second hatch were at 19°C. Smith et al. (2002) found newly hatched *J. edwardsii* phyllosoma from warmer water were significantly smaller. The warmer incubation temperature may have resulted in more energy being used for metabolism and less for development of the embryo. This difference at hatching between batches of *P. cygnus* larvae had become significant at the moult to stage II (Table 1). Larvae from the second hatch had developed significantly higher lipid levels (PL and total FA), suggesting the condition of larvae at hatch affected their condition into stage II, and possibly for further culture. However the relative contribution to the LC did not significantly differ between newly molted stage II larvae from either hatch (Table 2), indicating larvae were able to assume a similar % LC after just one moult.

4.5. Conclusions

Lipid was not a major component of the larvae and was not the major nutrient accumulated in fed larvae or used in starved larvae, however this does not imply lipid is of minor importance. Lipid was a greater component in stage II larvae than stage I larvae, possibly

indicating that lipid may assume a greater importance as larvae progress through developmental stages. Further studies could assess the importance of other nutrients (i.e. protein and carbohydrate) in phyllosoma development. The LC profile of the phyllosoma samples was dominated by PL. PL was also the main LC used during starvation and accounted for almost the entire increase in lipid in fed larvae. ST was the next most abundant LC which showed an increase in relative contribution in starved larvae, its retention possibly related to its important structural/function role. *Artemia* had high levels of TAG, however phyllosoma never accumulated TAG, suggesting phyllosoma are unable to absorb or accumulate TAG. The FA changes occurring in larvae appeared to be stage dependant, however HUFA, such as AA, EPA and DHA, were generally conserved (mainly in stage II larvae). Results from the current study will provide information for formulating diets for phyllosoma culture.

¡El Final!